

**PROTEIN DESIGN:
THE CRAFTING OF NOVEL MOTIFS IN PEPTIDE BACKBONES**

*A Thesis Submitted
in Partial Fulfilment of the Requirements
for the Degree of*
DOCTOR OF PHILOSOPHY

by
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to the
**DEPARTMENT OF CHEMISTRY
INDIAN INSTITUTE OF TECHNOLOGY, KANPUR**

OCTOBER, 1993

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STATEMENT

I hereby declare that the matter embodied in this thesis is the result of investigations carried out by me in the Department of Chemistry, Indian Institute of Technology, Kanpur, India, under the supervision of Professor S. Ranganathan.

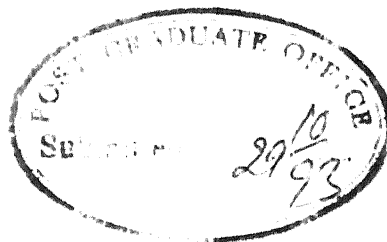
In keeping with the general practice of reporting scientific observations, due acknowledgements have been made wherever the work embodied is based on the findings of other investigators.

IIT Kanpur

October, 1993



NARENDRA KUMAR VAISH



CERTIFICATE

Certified that the work contained in this thesis, entitled, "PROTEIN DESIGN : THE CRAFTING OF NOVEL MOTIFS IN PEPTIDE BACKBONE" has been carried out by Mr. Narendra Kumar Vaish, under my supervision and the same has not been submitted elsewhere for a degree.

IIT Kanpur

October, 1993


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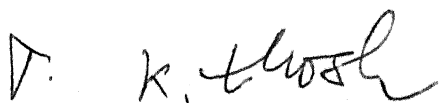
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CERTIFICATE OF COURSE WORK

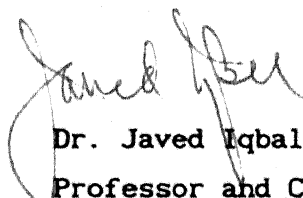
This is to certify that Mr. Narendra Kumar Vaish (Roll number 8920765) has satisfactorily completed all the courses required for the Ph.D. degree programme and obtained CPI of 9.33/10.0, these courses include:

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CHM 624 Modern Physical Methods in Chemistry
CHM 625 Principles of Physical Chemistry
CHM 641 Advanced Inorganic Chemistry
CHM 645 Principles of Inorganic Chemistry
CHM 681 Basic Biological Chemistry
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Mr. Narendra Kumar Vaish has successfully completed his Ph.D. qualifying examination on April 1991. He has also successfully presented his open seminar of the work embodied in this thesis.



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ACKNOWLEDGEMENTS

With great pleasure, I wish to express my deep sense of gratitude to Professor S. Ranganathan for his encouragement, invaluable and stimulating suggestions and creative guidance which greatly enhanced my interest in the subject. To work with him for the doctoral dissertation was a great privilege and pleasure for me. His endless creative ideas and critical insights helped me learn a lot both theoretical and practical aspects of Chemistry and Molecular Biology. His potential of working constantly and systematically round the clock will always remain a source of inspiration to me. I have great reverence for him.

Words are inadequate to express my indebtedness to Dr. (Mrs.) D. Ranganathan for her encouragement, advice and prudent guidance throughout my work. In true sense, she is my architect. It must have been an experience for her to transfer a novice like me into an enquiring mind with conflagrant desires. I feel privileged working with her. I also gratefully acknowledge my personal care taken by her. Here, I should not forget to mention about the delicious Allu-parathas provided by her on various occasions, giving me a homely atmosphere.

I am extremely grateful to Dr. G. P. Singh, Dr. Sujata and Dr. Kavita for helping and encouraging me at various stages of experimental techniques. I am very much thankful to Mr. Anand for help in computational molecular modelling and cheerful company during early stages of work.

I extend my thanks to all the faculty members of the Department of Chemistry, particularly to Profs. P. K. Bharadwaj, S. Manogaran, J. Iqbal and N. Sathyamurthy, for their generous help and encouragement.

I am very thankful to my colleagues Messers N. Jayaraman, B. K. Patel, S. K. George, N. Tamilarasu and D. Kundu, who always extended their

I express my thanks to Prof. D. Balasubramanian, Director CCMB, Hyderabad for providing me an opportunity to work at CCMB and Dr. R. Nagaraj (CCMB, Hyderabad) for helpful discussions.

I wish to express my thanks to Prof. P. T. Manoharan (IIT Madras) and Dr. I. Karle (Naval Research Laboratory, U.S.A.) for carrying out the X-ray analysis.

I am extremely thankful to Dr. Raja Roy (CDRI, Lucknow) for generously carrying out 400 MHz NMR spectral analysis and Dr. K. P. Madhusudanan (CDRI, Lucknow) for FAB mass spectral analysis.

I must not forget to mention cheerful company of my friends, Messers. Pramod, Arun, Alok, Tapan, Sujay, Tara, Kalyanaraman, Raghunathan, Madhava, Venkat, B. Dube, R. N. Tripathi, Puni, Samar, Kashi, Ilango, Dr. Samiran, Dr. Manbendra, Dr. Rameshwar, Dr. Pradeep, Dr. Ram Naresh, Dr. Sanjay and Dr. Govind who made my stay at IIT Kanpur a pleasurable one. I extend my thanks to my friends Dr. R.R. Misra, Anil, Subha, Sapana, Kalika, Nitin, Anirudh, Dushyant and Madhur for their moral support.

I thank Mr. Om Prakash for his assistance during my work.

My sincere thanks are due to Messers N. Ahmad, D. K. Kannaujia and Bhavsar for spectral and chemical analysis and RSIC unit of CDRI, Lucknow for Mass spectral analysis. My thanks are due to glass blowing, liquid nitrogen and chemistry workshop of IIT, Kanpur for their kind help.

I thank the staff members at the Department of Chemistry, Messers Bajpai, V. N. Katiyar, B. N. Shukla and Banwari for their ready help.

My sincere thanks to Dr. Naveen, Dr. Desh Deepak and Dr. Chauhan (Lucknow University, Lucknow) for genuine advice and help.

Last but not the least I wish to express my sincere thanks to all my family members for their consistent support and encouragement

PROTEIN DESIGN: THE CRAFTING OF NOVEL MOTIFS IN PEPTIDE BACKBONE

ABSTRACT

The genesis of the work reported in the thesis is related to the exploration of diverse facets associated with the generation of, in Nature, the primary amide functionality present at the carboxy terminus in the majority of biologically active peptides, hormones and neuropeptides from glycine residue at the C-terminus of their Gly extended precursors.

This reaction is catalyzed by the enzyme PAM (peptidylglycine α -amidating monooxygenase enzyme) via C^α -hydroxylation to a carbinolamide followed by retro-aminal process.

The thesis reports the successful chemical simulation of PAM action using C-terminal Ser/Thr esters via Ru(VIII) mediated C^α -C side chain bond scission. As in Nature, the simulation proceeds via carbinolamide, arising from addition of water to the initially formed, highly reactive, acylimine intermediate $[\text{CONH}(\text{CHROH})\text{COOMe} \longrightarrow \text{CON}=\text{CHCOOMe} \longrightarrow \text{CONHCH}(\text{OH})\text{COOMe} \longrightarrow \text{CONH}_2]$.

Noteworthy features of the terminal amidation mediated by Ru(VIII) species are, the stability of diverse N-protecting groups, the total unreactivity of the potentially susceptible side chains of Phe, Asn, Gln, Pro and nitro-Arg and the clean transformation of the methionine side chain to the sulfone.

In the first phase of this study, whilst 27 peptides containing Ser/Thr C-terminal esters cleanly afforded the expected terminal amides, 2 amongst the set gave CONHCOCOOME , arising from the further oxidation of the carbinolamide.

A mechanistic analysis of this anomaly showed that in the further

there exists a delicate balance between cleavage to primary amides and further oxidation to CONHCOCOX and that the major criterion for scission to amides is the requirement of anti-periplanar configuration of OH and CO around the C-N bond.

Molecular modelling studies brought out the fact that whereas a seven membered hydrogen bonded preferred conformation is not possible in the case of C-terminal Ser/Thr methyl esters, the same residues when located at the N-terminal and non-terminal locations would have this option, which would necessarily impede the required anti-periplanar configuration and therefore would promote oxidation over scission. Even more interesting is the rationalization that were the C-terminal Ser/Thr esters be replaced by the corresponding amides, a similar hydrogen bonded configuration for the carbinolamide becomes possible, thus leading to the prediction that these substrates would undergo oxidation in preference to scission.

Thus oxidation in preference to scission of the carbinolamide intermediates was anticipated in the Ru(VIII) mediated C^{α} -C side chain scission of the Ser/Thr residues located at the N-terminal or non-terminal sites on the one hand and for substrates having C-terminal Ser/Thr amide termini in place of C-terminal esters on the other. In the event, these expectations were fully realized leading to the delineation of novel methodologies for the placement of oxalamide units in the peptide backbone. Thus, 26 peptides having N-terminal Ser/Thr residues, 9 peptides where the Ser/Thr is placed in a non-terminal location and 6 peptides containing C-terminal Ser/Thr amides, were smoothly converted to oxalamides via the predicted oxidation of the carbinolamide intermediate.

A serendipitous finding is the oxidation of Z-Ala-Trp-OMe, with Ru(VIII) under usual conditions, to Z-Ala-NH₂, thus demonstrating Trp as a chemical equivalent in PAM mediated reactions. Tyr residues behave similarly. This unusual transformation has been realized on the basis of

addition provides a novel method for protein scission at Trp/Tyr sites.

The present study has also shown that when the Ser/Thr residues are placed contiguously, peptide scission, by hydrolysis of the initially formed extended oxalamide, takes place.

A significant outcome of the endeavours described above is the generation of, in a peptide backbone, the construct, CONHCOANHCH(R), which transforms a normal peptide into a retropeptide, the core of which being the oxalamide [NHCOCONH] unit. Here, the modulation of the COCO dihedral angle from one having a perfect C_2 symmetry to an orthogonal alignment has implications pertaining to transition state analogs associated with rotamase activity and immune suppression.

The crafting of the oxalamide unit into a peptide backbone has been accomplished and reported in the thesis. A range of oxalamido core elements of the type, $\text{MeO-A}_{aa}\text{-COCO-A}_{aa}\text{-OMe}$, have been made from C-protected amino acids and oxalyl chloride. Of particular interest here is the ready formation of such compounds involving Met, Tyr, Trp, Pro and N^α -protected Lys.

Interestingly, X-ray crystallographic studies have clearly brought out the modulation of the dihedral angle. Thus, whereas the retropeptide $(\text{MeO-Aib-CO})_2$ shows perfect C_2 symmetry (dihedral angle 180°), $(\text{MeO-Pro-CO})_2$ shows noteworthy deviation having a dihedral angle of 108° .

The bi-directional elongation of the core $\text{MeO-A}_{aa}\text{-COCO-A}_{aa}\text{-OMe}$ has been accomplished by two broad strategies. They were either hydrolysed to the corresponding acids and coupled with the appropriate partners using DCC/HOBt procedure or were transformed to the hydrazide and coupled by the azide route.

The core diacids readily form copper complexes harboring two metal atoms per substrate. The X-ray crystal structure of $(\text{Aib-COCO-Aib})\text{Cu}_2$ showed that the complex is a $(\text{Cu}_2\text{L})_n$ cluster, possessing a highly

the other blocks of the same lane as well as to that of neighboring lanes by carboxylato bridges. There are 2 metal atoms per block, locked in a dimeric fashion, with the dimer (constituting two symmetric halves of the block) having a centre of inversion at the bis-carbonyl unit of the core oxalamide motif. Each block provides 3 coordination sites for the metal, the fourth made available by a neighboring block via the carbonyl oxygen. When dissolved in DMF, the supramolecular assembly undergoes dissociation to individual blocks, wherein the fourth ligand site is occupied by the solvent.

The bi-directional elongation of the core motifs, by either of the routes referred to above, proceeds smoothly; further, the iterative aspect of the strategy has been demonstrated via further elongation of the first generation constructs. Bi-directional elongation with two residues resulted in the emergence of secondary structures, assessed on the basis of temperature dependent NMR and CD studies. Thus, $(\text{MeO-Gly-Ala-Leu-CO})_2$ possesses a C_2 symmetric secondary structural motif, chiefly arising from intramolecular hydrogen bonding involving the Leu-NH and the Gly-CO.

A practical fallout of the above endeavours is the discovery of an efficient and mild route for the transformation of the Ser residue, in peptides, to the dehydro-Ala units.

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Representation of amino acids and their derivatives:

- (1) All amino acids are represented by the standard three letter code eg. Ala-Gly represents a peptide formed from the amino acids alanine and glycine.
- (2) A symbol to the left and hyphenated is a N-terminal protection eg. Bz-Ala = N^αBenzoyl-alanine.
- (3) A symbol to the right and hyphenated is a C-terminal protection eg. Ala-OMe = methyl ester of alanine and Ala-OH represents simple alanine.
- (4) A symbol after the amino acid symbol and in parenthesis is a blocking group on the side chain eg. Asp(β-OMe) = β-COOMe aspartic acid.

Other abbreviations, used in the thesis, are as follows:

AcOH	: acetic acid
aq.	: aqueous
Boc	: tert-butyloxy carbonyl
BocN ₃	: tert-butyl azidoformate
Bz	: benzoyl
Bz-Cl	: benzoyl chloride
Bzl/Bn	: benzyl
CD	: circular dichroism
DCC	: dicyclohexyl carbodiimide
DCU	: dicyclohexyl urea
DMF	: dimethyl formamide
DMSO	: dimethyl sulfoxide
DNA	: deoxyribonucleic acid

DPPH	: dipicryl phosphoryl azide
Et ₃ N	: triethylamine
EPR	: electron paramagnetic resonance
EtOAc	: ethyl acetate
EtOH	: ethanol
FAB	: fast atom bombardment
HOBt	: hydroxybenzotriazole
ir	: infra red
MeCN	: acetonitril
MeOH	: methanol
mp.	: melting point
ms	: mass spectroscopy
nmr	: nuclear magnetic resonance
O- ^t Bu	: t-butyl ester
OBzl	: O-benzyl ester
OMe/OCH ₃	: O-methyl ester
ppm	: parts per million
rt	: room temperature
THF	: tetrahydrofuran
tlc	: thin layer chromatography
TMS	: tetramethylsilane
uv	: ultraviolet spectroscopy
VT	: variable temperature
Z	: benzyloxycarbonyl or carbobenzoxy

A. INTRODUCTION

The genesis of the work reported in the thesis is related to the exploration of diverse facets associated with the generation of, in Nature, the primary amide functionality present at the carboxy terminus in the majority of biologically active polypeptides, hormones and neuropeptides from a glycine residue at the C-terminus of their gly-extended precursors.

This reaction is catalyzed by the enzyme PAM (peptidyl glycine α -amidating mono-oxygenase enzyme) via C ^{α} -hydroxylation to a carbinol amide followed by a retro-aminal process.

The thesis reports the successful chemical simulation of PAM action using C-terminal Ser/Thr esters via Ru(VIII) mediated C-C bond scission. As in Nature, the simulation proceeds via carbinolamide, arising from addition of water to the initially formed highly reactive, acylimine intermediate.

A dramatic variant of the terminal α -amidation ensues when the carbinolamide intermediate preferentially undergoes oxidation, instead of the retro-aminal mode leading to peptide backbone modification arising from incorporation of the extended retropeptido oxalamide (-NHCOCONH-) unit. Extensive studies with a range of substrates have enabled the delineation of factors that contribute to the diversion.

Other novel findings have also been encountered in the course of the chemical simulation of PAM action, involving 70 peptide substrates.

The extended peptide segment, referred to above, has several interesting features. Thus, the NHCOCNH unit can be viewed as the core of a retropeptide and the modulation of the dihedral angle from one having a perfect C₂ symmetry to an orthogonal alignment has implications pertaining to transition state analogues associated with rotamase activity and immune suppression. The crafting of the retropeptido oxalamide unit into a

elements have been crafted from C-protected amino acids. X-ray crystallographic studies have clearly brought out the modulation of the -CO-CO- dihedral angle in these systems from 175° to 106° by imposition of steric constraints. Bi-directional elongation of the retropeptido oxalamide core has been shown and gradually leads to peptides having secondary structures. Preliminary X-ray crystallographic studies of the copper complexes of the terminal dicarboxy retropeptido oxalamide system has shown that it forms extended helical supramolecular structures arising from self-assembly of single units. The copper clusters thus formed have been demonstrated to effect DNA scission of supercoiled DNA pBR-322, as exemplified with six members of this class.

A practical fallout of the present work is the discovery of an efficient transformation of the Ser side chain, in peptide environments, to a dehydro Ala unit.

The systems and methodologies presented in the thesis will have ramifications right across the protein domain. Their potential in the modulation of protein function and protein design and their utility, in inter or intrastrand cross-linking, design of inhibitors, crafting of transition state mimics and the preparation of hormone antagonists would constitute some obvious options.

Belatedly, backbone modification as a strategy for protein design, has found recognition in recent times. A brief account of endeavours in this domain forms, appropriately, as the background to the work reported in the thesis.

B. BACKGROUND

The creation of novel molecular constellations having the potential for improved biological profiles is an area of immense importance in peptide chemistry.¹ This can be achieved by the structural modification of either the side chain² or the backbone elements³ in the peptide systems. Until recently, most of the synthetic efforts have been centred on the peptide side chains or have involved amino acid additions, deletions or substitutions rather than backbone modification of the parent peptide molecule. This may be due to the arduous synthetic endeavours involved in backbone replacements.

With the discovery of increasing number of biologically active peptides and the need to improve their biological half life, particularly with respect to their oral bioavailability and peptidase resistance, a flurry of activity is being witnessed in probing the role of protein backbone elements in relation to their biological functions.

Indeed, in the past 10-15 years, the design and synthesis of pseudopeptides or peptide isosteres⁴ containing numerous surrogates of the amide carbonyl (CO), of the amide nitrogen (NH) or of both groups (CONH) is emerging as a popular endeavour in peptide chemistry and is fast becoming the most attractive approach to overcome the poor stability, lack of oral absorption and marginal ability to cross the blood-brain barrier, in the use of peptides as therapeutic agents. The resulting pseudopeptides or isosteres have shown, for instance, as protease-resisting analogs and, in some cases, as potent protease inhibitors. Inherent in the concept of isosteric replacement of amide bonds in biologically active peptides is the postulate that it might be possible to modify one or more amide bonds in peptides such that the conformation and binding are maintained, but enzymatic hydrolysis is prevented.⁵

The peptide backbone comprises of three repeating elements, the

design, each of these elements has been subjected to replacement, either individually or in combination. The -CONH- group itself is also considered as a single replaceable element, although in this case the replacement effectively encompasses two adjacent amino acid residues. Each of the 3 backbone elements is considered separately in the following account.

In addition to the above, peptide backbone modification has been achieved by ingenious methods, notable amongst which are the insertion of constructs between the C^α and CONH on the one hand and between CONH and C^α on the other, insertion of heterocycles between C^α and CO unit and the use of natural or crafted side chains to cyclize on to the peptide backbone. These aspects have been briefly presented.

A comprehensive account of the replacements of backbone elements with a variety of isosteres forms the subject matter of this section and is presented below. Wherever possible, primary sources have been consulted; in all the other cases, individual citation is from secondary channels.

1. Modification of the Amide NH in the Peptide Backbone

As presented in table 1, six NH surrogates have been reported which include, the most common N-alkyl isosteres (NR), depsipeptides (COO), thio esters (COS), ketomethylenes ($COCH_2$), and N-hydroxy (-N(OH)-) isosteres. The modifications have been carried out in enkephalins, LH-RH, bradykinin analogs, substance P, ACE inhibitors, Renin inhibitors and Angiotensin analogs. Interestingly, the modified isosteres, in most cases retain the biological activity, even in the case of N-alkylated isosteres where the H-bonding capability is lost due to NH substitution.

2. Modification of the Amide CO in the Peptide Backbone

Table 2 presents the isosteres at amide carbonyl of the peptide bond. The examples illustrated in this table include small peptide substrates, such as, analogs of enkephalins, penstatine, statine, oxytocin, aspartame and inhibitors of ACE, HIV protease and human renin.

$P(O)OR$, $CHOHCH_2$ (racemic), $COCH_2$, $C(OH)(CH_3)CH_2$ (racemic), CH_2SO_2 , $\bar{P}O_2CH_2NH$, $C^\alpha-NH$ inserts, $CH(OH)CH_2CO$ (racemic), $CO(CH_2)_3CO$ and $CH=CHCO$. These replacements introduce multiple variations in the reactivity profile and also impart increased flexibility, greater lipophilicity and alter the conformational profile of ordered structures containing H-bonded features.

3. Modification of the Amide (CONH) bond in the Peptide Backbone

Driven by the quest to develop novel and potent protease inhibitors using the concept of isosteric replacement of amide bond in small peptides, numerous amide bond isosteres have been reported in recent years (Table 3). Amongst the several mimics which have appeared in the literature, the trans C-C double bond appears to be the most suitable moiety to mimic the linkage in terms of geometry and bond angles and length. Additionally, unlike the -CONH- bond, which has some degree of flexibility and possesses H-bonding capability, the trans double bond is expected to fix the replaced peptide linkage in a trans conformation and eliminate its H-bonding properties. Therefore, trans double bond isostere analogs can provide valuable information concerning the role of an amide bond at a specific site in a peptide and therefore is considered an ideal replacement for amide group. The unit $[CH_E=CH]$ has been incorporated within a large number of renin inhibitors, enkephalins and substance P analogs and a host of dipeptide and tripeptide enzyme inhibitors.

The successful preparation of CH_2S isostere was first reported by Spatola in enkephalin analogs⁵⁴ and later this unit was incorporated into the cyclic peptide cyclo[Gly-Pro-Gly-D-Phe-Pro]. The resulting conformation was found to be compatible with the original backbone conformation of the cyclic peptide wherein both β - and γ -turn features were found to be conserved⁵⁷. Other examples of replacement cited in Table 3 include, $CONH \rightarrow CF_E=CH$, $CONH \rightarrow CH_2O$, $CONH \rightarrow NHCO$ (with and without

(mixtures), $\text{CONH} \longrightarrow \text{CH}(\text{OH})\text{CH}=\text{CHCO}$ (mixtures), $\text{CONH} \longrightarrow (\text{CHOH})_3\text{CO}$
 (mixtures), $\text{CONH} \longrightarrow \text{CH}_2\text{SO}$, $\text{CONH} \longrightarrow \text{CH}_2\text{SO}_2$, $\text{CONH} \longrightarrow \text{CH} \begin{array}{c} \diagup \text{O} \diagdown \\ \text{CH} \end{array} \text{CH}$, $\text{CONH} \longrightarrow$
 $\text{COCH}_2\text{NCH}_3$, $\text{CONH} \longrightarrow \text{CH}=\text{CH}-\text{CH}_2$ and $\text{CONH} \longrightarrow \text{NHCONH}$. In several cases the
 biological profile of the modified peptides have been extensively studied.
 They do exhibit an inhibitor profile and many of the examples cited in
 Table 3 have potential as therapeutic agents. This feature of "secondary
 structure peptido mimetics" is expected to find applications in the
 development of enzyme inhibitors.

4. Modification at α -Carbon

Only a handful of replacements have been reported at α -carbon of
 the peptides.³ This is understandable because of the synthetic arduous
 involved in such replacements. The most commonly used replacement appears
 to be the alkyl substitution which includes cyclopropyls also. The
 presence of alkyl substituents at α -carbon is known to modify the
 conformational and biological behaviour of peptides in general and may be
 useful to design altered biological analogs.

Judging from the number of review articles¹⁰ which, have
 appeared in the last 10-15 years, dehydroamino acids (Δ -amino acids)
 appear to be one of the most promising new-generation backbone
 replacements. With sp^2 hybridization at the α -carbon, peptides gain a more
 rigid side chain orientation in place of a chiral centre. The near
 planarity of the group, and the altered conjugated electronic character
 may contribute to its reported resistance to enzymatic degradation. This
 property was confirmed with several examples, notable amongst which are,
 angiotensin, bradykinin, aspartame, LH-RH, enkephalin and their analogs
 (See Table 4).

5. Modification by Insertion of Constructs between C^α and NHCO

Notable amongst the three examples cited in Table 5, is the
 insertion of the $\text{HC}=\text{CH}$ unit, to provide extended amide conjugation. The

6. Modification by Insertion of Construct between CONH and C^α

An interesting modification of this class is the insertion of CH₂CH₂S at all sites in the cyclic peptide, Cyclo[Phe-Phe-Phe] (Table 6). The other illustrations are insertion of oxo (-O-)(ie CONH-O-, aminoxy).

7. Modification by Insertion of Construct between C^α and CO

Oxazolidine and imidazolidine have been inserted to provide peptides with totally altered conformation (Table 7).

8. Modification by Cyclization to Backbone

Structures arising from cyclization of π extended peptide NH to the C^α sites provide excellent β pleated sheet mimics. Another reaction of good potential is the linking of the peptide NH with side chains of amino acids (Table 8).

The above account should highlight the importance of peptide backbone modification in modulating and stabilizing of biologically important peptides.

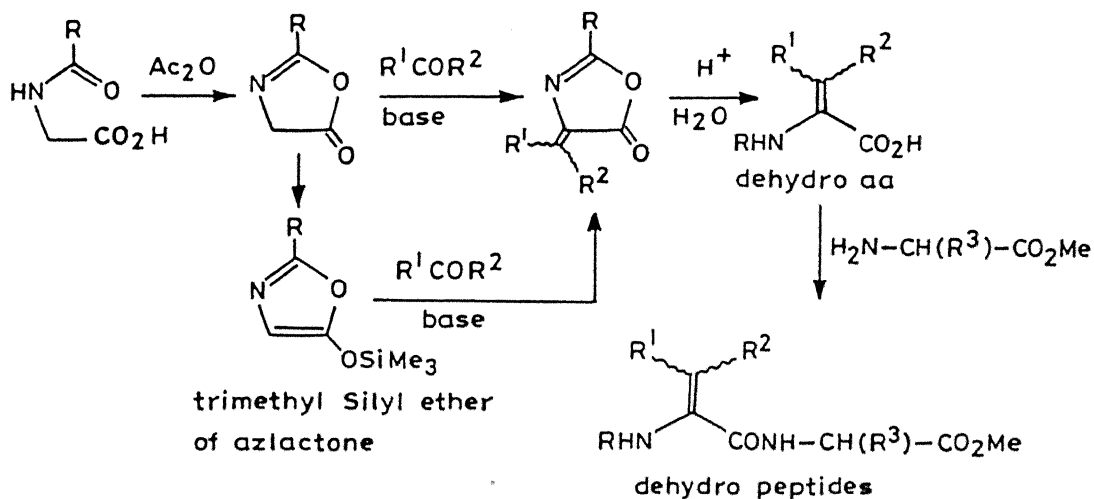
In addition to the above, it was considered appropriate to outline the handful of methods hitherto available for the generation of the Δ Ala units, to provide a background to our own endeavours which resulted in a very satisfactory method for the preparation of this unit in intact peptides (Section C).

Chart-1 shows various strategies employed for the synthesis of Δ -amino acids. Each method has its own advantages and disadvantages. For example, N-chlorination and dehydrohalogenation (Method 5) to the α,β stabilized olefin can be used when only one amide NH is present, as in the synthesis of a TRF analog.¹¹ In general, however, the methods involving elimination of β -functionalized precursors¹² (Methods 2,6), direct oxidation of aromatic dipeptide azlactones with DDQ¹³ or a novel selenium based protecting group¹⁴ that permits facile chain extension of the dehydro-synthon containing groups seem to be promising.

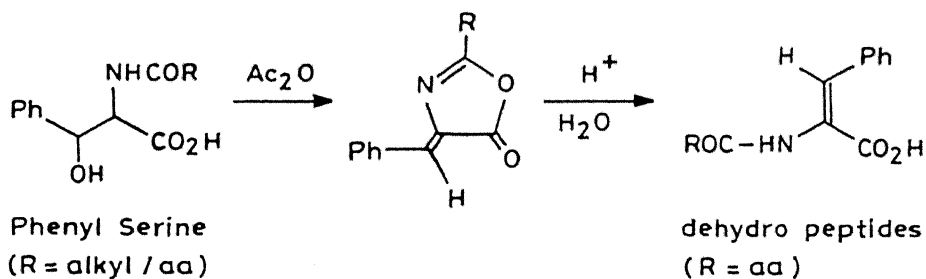
Synthetic methods to generate dehydro units in peptides

(1) Azlactone method.¹⁰⁰

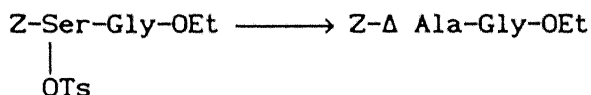
(a)



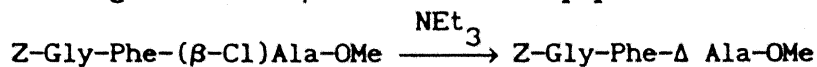
(b)¹⁰¹



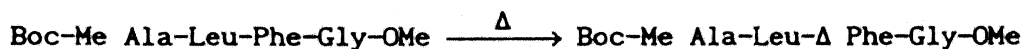
(2) By base catalysed β -elimination of OTs from O-tosyl serine derivatives.¹⁰²



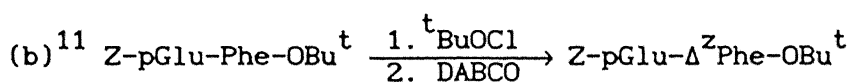
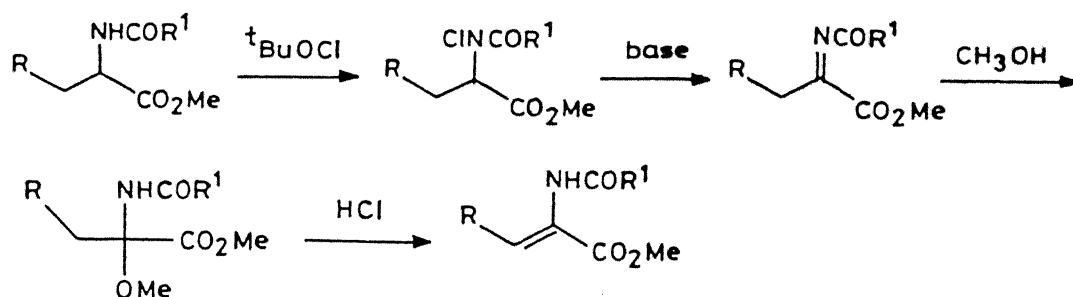
(3) By dehydrohalogenation of β -chloroalanine peptides.¹⁰³



(4) By pyrolysis of S-benzyl cysteine sulfoxides.¹⁰⁴



(5) By dehydrohalogenation of N-chlorinated amino acids.

(a)¹⁰⁵

TRF analog

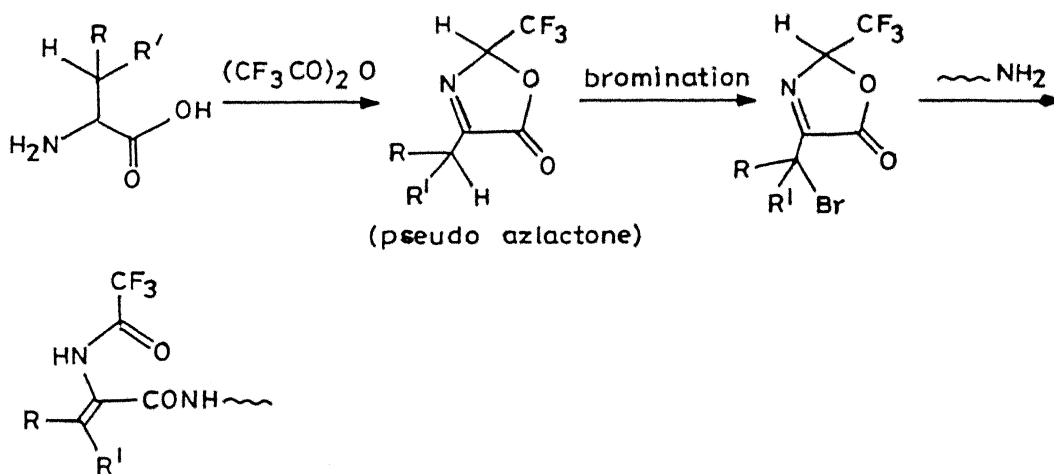
(6)¹²

Table 1: Modification of Amide NH in the Peptide Backbone [-NH \longrightarrow X]

Entry	X	Backbone modification (underlined)	Examples	No. of units replaced	Ref.
1	N-CH ₃ [N-Methyl]	-NH-CHR- <u>CO-N(CH₃)</u> -CHR'-CO-	Enkephalins, LH-RH, Substance P, analogs	1 1 2	15, 16 17 18
2	-CH ₂ [Ketome- thylene]	Ph-CO-NH-CH(CH ₂ Ph)- <u>CO-CH₂</u> - CH ₂ -CO-Pro	ACE inhi- bitors	1	19, 20
		Ph-CO-NH-CH(CH ₂ Ph)- <u>CO-CH₂</u> - CH(CH ₃)-CO-Pro	ACE inhi- bitors	1	21
		R-NH-CH(CH ₂ CH(Me) ₂)- <u>CO-CH₂</u> - CH(CH ₃)-CO-Iaa R=Iva-Val-Val (Iva = isovaleryl) Iaa = isoamylamide	Peptide - analogs	1	22
		Boc-NH-CH(CH ₂ Ph)- <u>CO-CH₂</u> -CH(CH ₂ - CH(Me) ₂)-CO-Sta-Leu-NHCH ₂ Ph Sta = 4(S)-amino-3(S)-hydroxy- 6-methylheptanoic acid	Renin - inhibitors	1	23
		Z-Phe- <u>CO-CH₂</u> -Ala Z-Ala- <u>CO-CH₂</u> -Ala Z-Ala- <u>CO-CH₂</u> -Asp	Enzyme - substrates	1	24

3.	-O	-NH-CHR- <u>CO-O</u> -CHR'-CO-	Angiotensin,	-	25
	[Ester]		Bradykinin,	1	26
			analogs		
4	-S	-NH-CHR- <u>CO-S</u> -CHR'-CO-	Enzyme subs-	1	27
	[Thioesters]		trates		
5	-N(OH)	-NH-CHR- <u>CO-N(OH)</u> -CHR'-CO-			
	[N-hydroxy]				
		Phthaloyl-NH-CH ₂ - <u>CO-N(OH)</u> -	Dipeptide -	1	69
		CH(CH ₃)COO ^t Bu	analogs		

Table 2: Modifications of the Amide Carbonyl (CO) in the Peptide Backbone

[CO \longrightarrow X]

Entry	X	Backbone modification (underlined)	Examples	No. of units replaced	Ref.
1	CH ₂ [Reduced carbonyl]	-NH-CHR- <u>CH₂</u> -NH-CHR'-CO-	Enkephalins	1	28
		Boc-Pro- <u>CH₂</u> -NH-Leu-Gly-NH ₂	Tripeptide - analogs	1	29
		Tyr-D-Ala-Phe- <u>CH₂</u> -NH-Gly-NH ₂	Dermorphine	1	30
		Tyr-D-Ala- <u>CH₂</u> -NH-Phe-Gly-NH ₂	tetrapeptides		
		Tyr- <u>CH₂</u> -NH-D-Ala-Phe-Gly-NH ₂			
		Boc-Gly- <u>CH₂</u> -NH-Leu-OMe	Dipeptide	1	31
		Z-Gly- <u>CH₂</u> -NH-Leu-OMe	isosteres		
		Z-Leu- <u>CH₂</u> -NH-Leu-OMe			
		$\overbrace{\text{Mpa-Tyr-X-Gln-Asn-Cys-Pro-NH-}}^{\text{CH(CH}_2\text{-CH(Me)}_2\text{)-CH}_2\text{-NH-Gly-NH}_2}$	Analog of	1	32
		X=Ile, Phe	des-amino oxy- tocin and des- amino-oxypressin		
		Mpa=3 mercaptopropionic acid			
			Renin inhibi- tor octapeptide	-	33
		Boc-Phe- <u>CH₂</u> -NH-Phe-Sta-Leu-NH- CH ₂ -Ph	Renin - inhibitors	1	23

		$R_1\text{-NH-CHR}_2\text{-}\underline{\text{CH}_2\text{-NH-CHR}_3\text{-CO}_2R_4}$ $R_1=\text{Z/Boc}; R_2, R_3, R_4=\text{alkyl, aryl}$	Dipeptide - isosteres	1	34
2	CS [Thioamide]	$\text{-NH-CHR-}\underline{\text{CS-NH-CHR}'}\text{-CO-}$	Aspartame - analog	1	35
		$\text{Bz-NH-CH}_2\text{-CO-NH-CH}_2\text{-}\underline{\text{CS-NH-CH(CH}_2\text{Ph)-COOH}}$	Carboxypepti- dase substrate	1	36
			Oxytocin - analog	1	37
		$R_1\text{-NH-CHR}_2\text{-}\underline{\text{CS-NH-CHR}_3\text{-CO}_2R_4}$ $R_1=\text{Z/Boc}; R_2, R_3, R_4=\text{alkyl, aryl}$	Endothiodipe- ptide analogs	1	34, 38
		$\text{H}_2\text{N-Tyr(OBzl)-Gly-}\underline{\text{CS-NH-Gly-Phe-Leu-OR}}$	Enkephalin - analog	1	39
3	PO(OR')- [Phospho- amide]	$\text{R}'\text{OOC-CH}_2\text{-NH-CH}_2\text{-}\underline{\text{PO(OR}')\text{-NH-CHR-COOR}'}$ $\text{R=H; R}'=\text{OEt}$	Di and Tri- peptide analogs	1	40
4	[*] (CHOH-CH ₂)- [hydroxy eth- ylene] racemic	$\text{-NH-CHR-}\underline{\text{CHOH-CH}_2\text{-NH-CHR}'}\text{-CO-}$ $\text{Ac-Ser-Leu-Asn-NH-CH(CH}_2\text{Ph)-}\underline{\text{CHOH-CH}_2\text{-Pro-Ile-Val-OMe}}$ $\text{H}_2\text{N-CH(CH}_2\text{CH(CH}_3\text{)}_2\text{)-}\underline{\text{CHOH-CH}_2\text{-Leu-Ile-Phe-OMe}}$	HIV-Protease inhibitors Enkephalin - analog	1	73 74

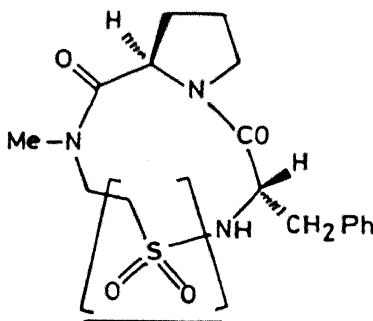
	$\text{Bz-NH-CH(CH}_2\text{Ph)-}\underline{\text{CHOH-CH}_2\text{-NH-}}$ $\text{CH(CH}_3\text{)-CO-Pro-OH}$	ACE inhi- bitors	1	76
5	COCH_2	$-\text{NH-CHR-}\underline{\text{COCH}_2\text{-NH-CHR'}}-\text{CO-}$		
	$\text{Bz-NH-CH(CH}_2\text{Ph)-}\underline{\text{COCH}_2\text{-NH-CHR-}}$ CO-Pro-OH	ACE inhi- bitors	1	77
6	$-\overset{*}{\text{C}}(\text{OH})(\text{CH}_3)-$ CH_2- Racemic	$-\text{NH-CHR-}\underline{\text{C}(\text{OH})(\text{CH}_3)\text{-CH}_2\text{-NH-CHR'}}-\text{CO-}$ $\text{Bz-CH(CH}_2\text{Ph)-}\underline{\text{C}(\text{OH})(\text{CH}_3)\text{-CH}_2\text{-NH-}}$ $\text{CH(CH}_3\text{)-CO-Pro-OR}$	ACE inhi- bitors	1 78
7	$\text{CH}_2\text{-SO}_2$		Cyclic pep- tide Analog	1 79
8	$\text{PO}_2\text{CH}_2\text{NH}$	$\text{Ac-Ser-Leu-Asn-NH(CH}_2\text{Ph)-}$ $\underline{\text{PO}_2\text{-CH}_2\text{-Pro}^+}\text{-Ile-Val-OMe}$	HIV protease inhibitors	1 80

Table 3: Modifications of the Amide CONH bond in the Peptide Backbone

(CONH \longrightarrow X)

Entry	X	Backbone modification (underlined)	Examples	No. of units replaced	Ref.
1	CH=CH E	-NH-CHR- <u>CH=CH</u> -CHR'-CO-	Double bond - isosteres of dipeptides	1	41,45 46,47 49,50
		Boc-Phe- <u>CH=CH</u> -Gly-Sta-Leu- NHCH ₂ Ph	Renin - inhibitors	1	23
		Tyr- <u>CH=CH</u> -Gly-Gly-Phe-Leu	Enkephalin and substance P analogs	1	42, 43,44
		Z-Phe-Gly- <u>CH=CH</u> -(R,S)Ala	Tripeptide - isosteres	1	48
2	C(F)=CH E	R-NH-Phe- <u>CF=CH</u> -Gly R = Boc/Fmoc/Z	Dipeptide and substance P analog	1	51
3	CH ₂ -O	-NH-CHR- <u>CH₂-O</u> -CHR'-CO-	Dipeptide - isosteres	1	52
4	CH ₂ -S	-NH-CHR- <u>CH₂-S</u> -CHR'-CO-	ACE inhi- bitors	1	53
		H-Tyr- <u>CH₂-S</u> -Gly-Gly-Phe-Leu-OH	Enkephalin -	1	54

LH-RH - 1 55

analogs

Iva-Phe-CH₂-S-Phe-Sta-Ala-Sta-
NHCH₂Ph

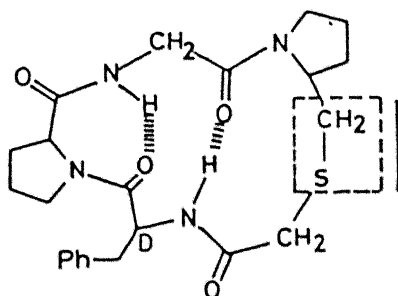
Renin - 1 23

inhibitors

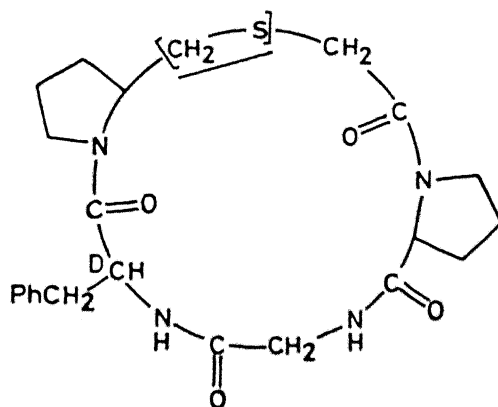
[Leu-CH₂-S-Gly⁸⁻⁹]₂OXT

Oxytocin - 1 56

analogs



Cyclic pep- 1 57a
tide analogs



Cyclic pep- 1 57b
tide analogs

5 NH-CO
retro

-NH-CHR-NH-CO-CHR-CO-NH-

Aspartame - 1 58
analogs

LH-RH - - 59
analogs

Bradykinin- 5 60

	$H_2N-CH(CH_2Ph)-\underline{NH-CO}-CH(CH_3)-COOH$	Retro-dipept-	1	61
		ides, enkepha-		
		linase inhib-		
		itors		
	$Boc-NH-CH(CH_3)-\underline{NH-CO}-CH(CH_3)-COOR$	Retrodipept-	1	62
		ides		
6	$NH-CO$ retro-inverso $S \rightarrow R$	$\begin{matrix} * \\ R \end{matrix} CH(R_1)-\underline{NH-CO}-\begin{matrix} * \\ R \end{matrix} CH(R_2)-\underline{NH-CO}-\begin{matrix} * \\ R \end{matrix} CH(R_3)-$ retro-inverso peptides	Angiotensin - analogs LRF analogs Somatostatin analogs Enkephalin - analogs	all R 63 63 63 63

8	NH-CS retro thio	$R^1HN-CHR-CO-NH-CHR^2-\underline{NH-CS-CHR^3}-CO_2R^4$ $R^1 = Z, Boc, Ac; R^2 = Me, Bz, {}^1Pr;$ $R^3 = H, Me, Bz, {}^1Pr; R^4 = Et, Ph$	Retro thio dipeptides	1	62
9	NH-SO ₂ retro sulfonamide	Boc-Pro-Leu- <u>NH-SO₂</u> -Gly-NH ₂	Tripeptide - analogs	1	67
10	CH ₂ -CH ₂	-NH-CHR- <u>CH₂-CH₂</u> -CHR'-CO-	Enkephalin - analogs	-	28
		Boc-Phe- <u>CH₂-CH₂</u> -Gly-Sta-Leu-NHCH ₂ Ph	Renin - inhibitors	1	23
11	CHOH-CH ₂ racemic	-NH-CHR- <u>CHOH-CH₂</u> -CHR'-CO-			
		Boc-phe- <u>CHOH-CH₂</u> -Gly-Sta-Leu-NHCH ₂ Ph	Renin - inhibitors	1	33,23
		R-NH-CH(CH ₂ CHMe ₂)- <u>CHOH-CH₂</u> -CH(CH ₃)-CO-Iaa R=Iva-Val-Val	Pepstatin - analogs	1	22
		Boc-NH-CH(CH ₂ Ph)- <u>CHOH-CH₂</u> -CH(CH ₂ Ph)-CONH-CH ₂ Ph	The core unit of potent HIV-1 protease inhibitors	1	70
		R'-CONH-CH(CH ₂ C ₆ H ₁₁)- <u>CHOH-CH₂</u> -		1	71

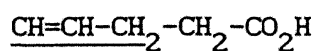
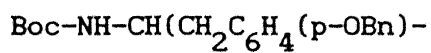
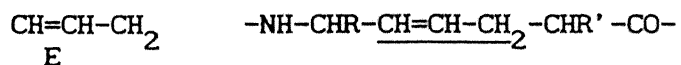
- 12 CHOH-CHOH $-\text{NH-CHR-}\underline{\text{CHOH-CHOH}}\text{-CHR'}-\text{CO-}$
 racemic
 $\text{Boc-Phe-His-NH-CH(CH}_2\text{C}_6\text{H}_{11}\text{)-}$ Renin - 1 72,23
 $\underline{\text{CHOH-CHOH-R}}$ inhibitors
 R=H, isobutyl, isopropyl or ethyl
- 13 CHOH-CH=CH- $-\text{NH-CHR-}\underline{\text{CHOH-CH=CH-CO-CHR'}}-\text{CO-}$ Renin - 1 23
 CO inhibitors
 racemic
- 14 CHOH-CHOH- $-\text{NH-CHR-}\underline{\text{CHOH-CHOH-CHOH-CO-CHR'}}-\text{CO-}$ Renin - 1 23
 CHOH-CO CO- inhibitors
 racemic
- 15 $\text{CH}_2\text{-SO}$ $-\text{NH-CHR-}\underline{\text{CH}_2\text{-SO-CHR'}}-\text{CO-}$ Renin - 1 23
 inhibitors
- 16 $\text{CH}_2\text{-SO}_2$ $-\text{NH-CHR-}\underline{\text{CH}_2\text{-SO}_2\text{-CHR'}}-\text{CO-}$ Renin - 1 23
 inhibitors
- 17 $\text{-CH}\overset{\text{O}}{\triangle}\text{-CH-}$ $-\text{NH-CHR-}\underline{\text{CH}\overset{\text{O}}{\triangle}\text{-CH-CHR'}}-\text{CO-}$
 $\text{Boc-NH-CH(CH}_2\text{Ph)-}\underline{\text{CH}\overset{\text{O}}{\triangle}\text{-CH-}}$ Dipeptido- 1 81
 $\text{CH}_2\text{-CO}_2\text{Me}$ mimetics
- 18 $\text{COCH}_2\text{N(CH}_3\text{)}$ $-\text{NH-CHR-}\underline{\text{COCH}_2\text{N(CH}_3\text{)-CHR'}}-\text{CO-}$



ACE inhi-

1

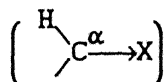
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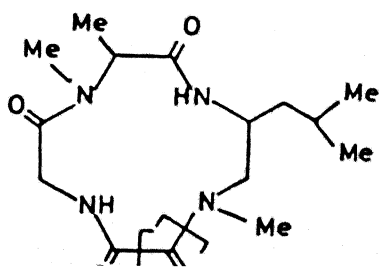
Dipeptide - 1 84
analogs



Angiotensin - 1 99
analogs

Table 4: Modifications of the Peptide Backbone Involving the α -Carbon

entry	X	Backbone modification (underlined)	Examples	No. of units replaced	Ref.
1	$\text{C} \begin{array}{l} \diagup \\ \diagdown \end{array}$ [α, α -disubstituted]	$-\text{NH}-\underline{\text{C}(\text{R}^1)(\text{R}^2)}-\text{CO}-$	Angiotensin, Bradykinin analogs	1	3
2	N- [α -aza]	$-\text{NH}-\underline{\text{N}(\text{R})}-\text{CO}-$	Enkephalin - Luliberin - analogs	1	85 86
3	$-\text{C}(=\text{CR}^1\text{R}^2)-$ [dehydro, Δ]	$-\text{NH}-\underline{\text{C}(=\text{CR}^1\text{R}^2)}-\text{CO}-$			10
		Boc-Asp(β -OBzl)- Δ Phe-OMe	Aspartame - analogs	1	87
		Z-Gly-Gly-Phe- Δ^Z Phe-Ala-OH	Enkephalins - analogs	1	88
		pGlu- Δ^Z Phe-Pro-NH ₂	TRF - analogs	1	89
			Δ -Ala Trypsin	1	90
			Tentoxin and its analogs	1	92



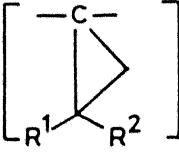
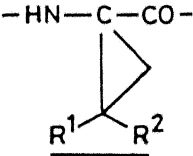
		Enkephalin -	-	93
		analogs		
	$[\text{Glp}^6, \Delta \text{Phe}^7] \text{SP}_{6-11}$	Substance P -	1	94
		analogs		
	$[\Delta \text{Phe}^4] \text{Angiotensin II}$	Angiotensin -	1	95
		analogs		
	Cyclo-(Pro-Phe- Δ^Z Phe-D-Trp-Lys-Thr-Phe)	Somatostatin analogs	1	96
4	-B [α -bora]	NH- <u>B(R)</u> -CO-	Boraglycine	- 16
5	-C(Br)- [α -bromo]	-NH- <u>CR(Br)</u> -CO-		
		PhCO-NH- <u>CH(Br)</u> -CONH-CHR-CO ₂ Me	Dipeptide -	1 91
			isosteres	
6	  [cyclopropylogs]	Enkephalin -	-	97,98
		analogs		

Table 5. Modifications by Insertion of Construct between C^α and CONH

Entry	construct	Backbone modification (underlined)	Example	No. of units replaced	Ref.
1	CHOH-CH ₂	α -NH-CHR- <u>CHOH-CH₂</u> -CO-NH-CHR ¹ -CO-	Pepstatin and statin analogs	1	75
2	CO(CH ₂) ₃	α Bz-NH-CH(CH ₂ Ph)- <u>CO(CH₂)₃</u> -CO- Pro-OH	ACE - inhibitors	1	77
3	CH=CH E	α Boc-NH-CH(CHMe ₂)- <u>CH=CH</u> -CO-NH- α CH(Me)-CO ₂ Me		1	82

Table 6. Modifications by Insertion of Construct between CONH and C^α

Entry	Construct	Backbone modification (underlined)	Example	No. of units replaced	Ref.
1	-O- [oxo]	-NH-CHR- <u>CO-NH-O</u> -CHR'-CO-	Aspartame - aminoxy analog	1	68
		Trp-Met-Asp(β-OH)- <u>CO-NH-O</u> - Phe-NH ₂	Aminoxy ana- log of Gastrio- tetrapeptide - amide	1	68
2	CH ₂ CH ₂ S	$ \begin{array}{c} \text{CH}_2\text{Ph} \qquad \qquad \text{CH}_2\text{Ph} \\ \qquad \qquad \qquad \\ \alpha \text{ CH-CO-NH-(CH}_2\text{)}_2\text{-S-CH } \alpha \\ \qquad \qquad \qquad \\ \text{S} \qquad \qquad \qquad \text{CO} \\ \qquad \qquad \qquad \\ \text{(CH}_2\text{)}_2 \alpha \qquad \qquad \text{NH} \\ \qquad \qquad \qquad \\ \text{NH-CO-CH-S(CH}_2\text{)}_2\text{-NH} \\ \qquad \qquad \qquad \\ \text{CH}_2\text{Ph} \qquad \qquad \text{CH}_2\text{Ph} \end{array} $	Cyclic pep- tide analogs	3	83

Table 7. Modifications by Insertion of Construct between C^α and C⁰

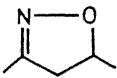
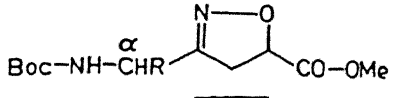
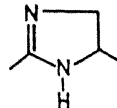
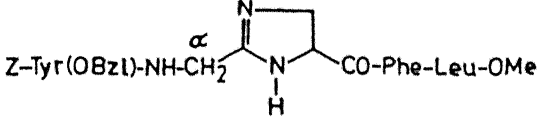
Entry	Construct	Backbone modification (underlined)	Example	No. of units replaced	Ref.
1		 $R = \text{CH}_2\text{OH}; \text{CH}(\text{CH}_3)\text{OH}; \text{CH}_2\text{CHMe}_2;$ CH_2Ph	Enzyme - inhibitors	1	6
2			Enkephalin - analogs	1	9

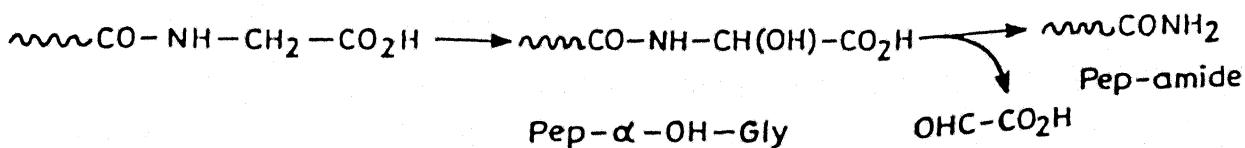
Table 8. Modifications by Cyclization to Backbone

Entry	Type	Backbone modification	Example	No. of units replaced	Ref.
1	$\text{NH} \rightarrow \text{C}^\alpha$		β -pleated - sheet mimic	3	7
2	$\text{C}^\alpha \text{ side chain} \rightarrow \text{NH}$		hGH(7-13) - analog	1	8

C. PRESENT WORK

The selective scission of proteins arising from ribosomal translation to smaller fragments is an important post-translational operation that takes place across the cellular membrane. An obvious advantage of this strategy is to reduce the burden on the information system pertaining to the biosynthesis of small peptide elements. However, it appears that this advantage has to be weighed against the necessary biosynthesis of enzymes that are needed to bring about the selective rupture of a large protein. The genesis of the present work is related to the understanding of one of the most intriguing post-translational changes referred to above wherein a host of biologically active polypeptides, hormones and neuro-peptides are crafted involving a two pronged operation, namely, selective recognition and cleavage leading to a C-terminal glycine residue and the C^α-N scission which in sum would transform the C-terminal Gly to an amide unit. Two enzyme systems are involved in this change. The first is associated with the recognition and cleavage at the Gly residue. Very little is known about this interesting reaction excepting for the fact that the reaction requires the presense of basic sequences such as arginine and lysine, to target at the specific Gly C-terminal peptide bond. In sharp contrast, pathways involved in the transformation of C-terminal Gly to the -NH₂ unit, catalysed by peptidylglycine α-amidating monooxygenase enzyme (PAM) has been well studied. There is a general agreement that the PAM action involves selective C^α-hydroxylation followed by the normal non-enzymatic fragmentation of the resulting carbinolamide¹⁷⁵ (Scheme C.1).

Scheme C.1



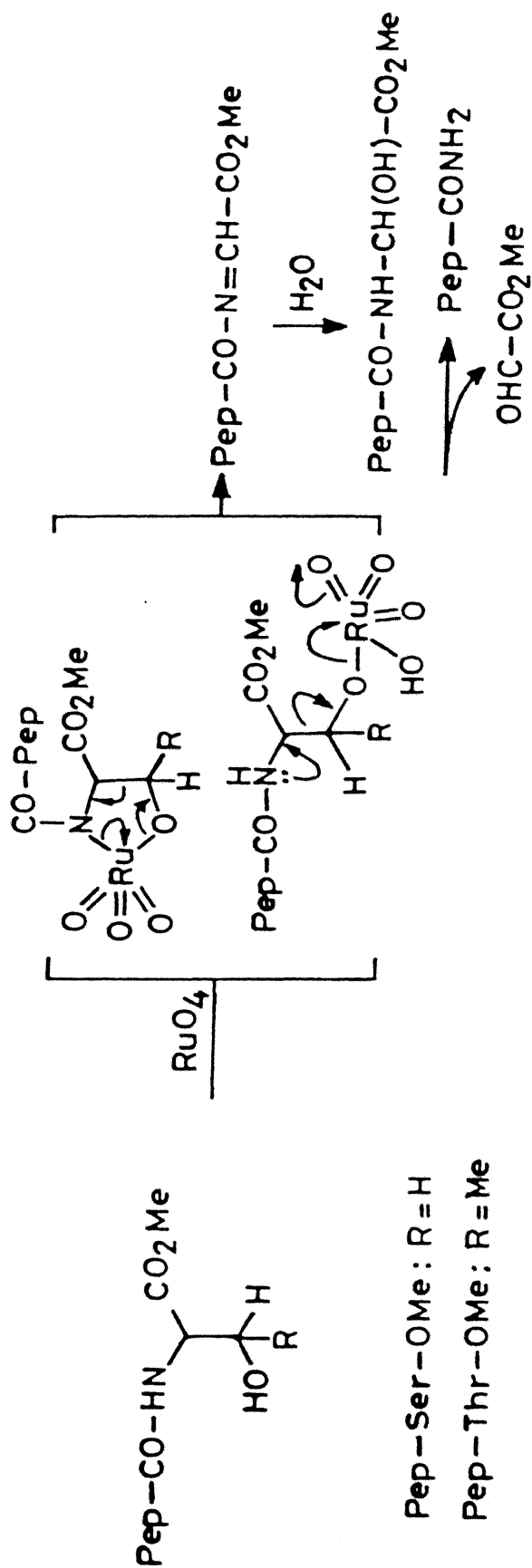
As could be seen from Scheme C.1 the α -hydroxylation of Gly residue would require, regardless of finer aspects of reaction mechanisms, the rupture of the C^α -H bond. It was considered logical therefore that the same key carbinolamide intermediate would arise by a C^α -C side chain bond scission. Naturally, the side chains that would be more appropriate here would be that of serine and threonine. Thus, in a chemical sense C-terminal Ser/Thr residues could be considered as glycine equivalents in terms of PAM reaction profile. In the event it was found that the desired transformation of C-terminal Ser/Thr residues to the $-NH_2$ unit, involving C-C bond scission could be readily brought about using *in situ* generated Ru(VIII) species (Scheme C.2).

C-terminal amidation mediated by PAM involving C-terminal Gly unit is compared in Scheme C.3 with the chemical methodology which achieves the same end result from a C-terminal Ser/Thr precursor.

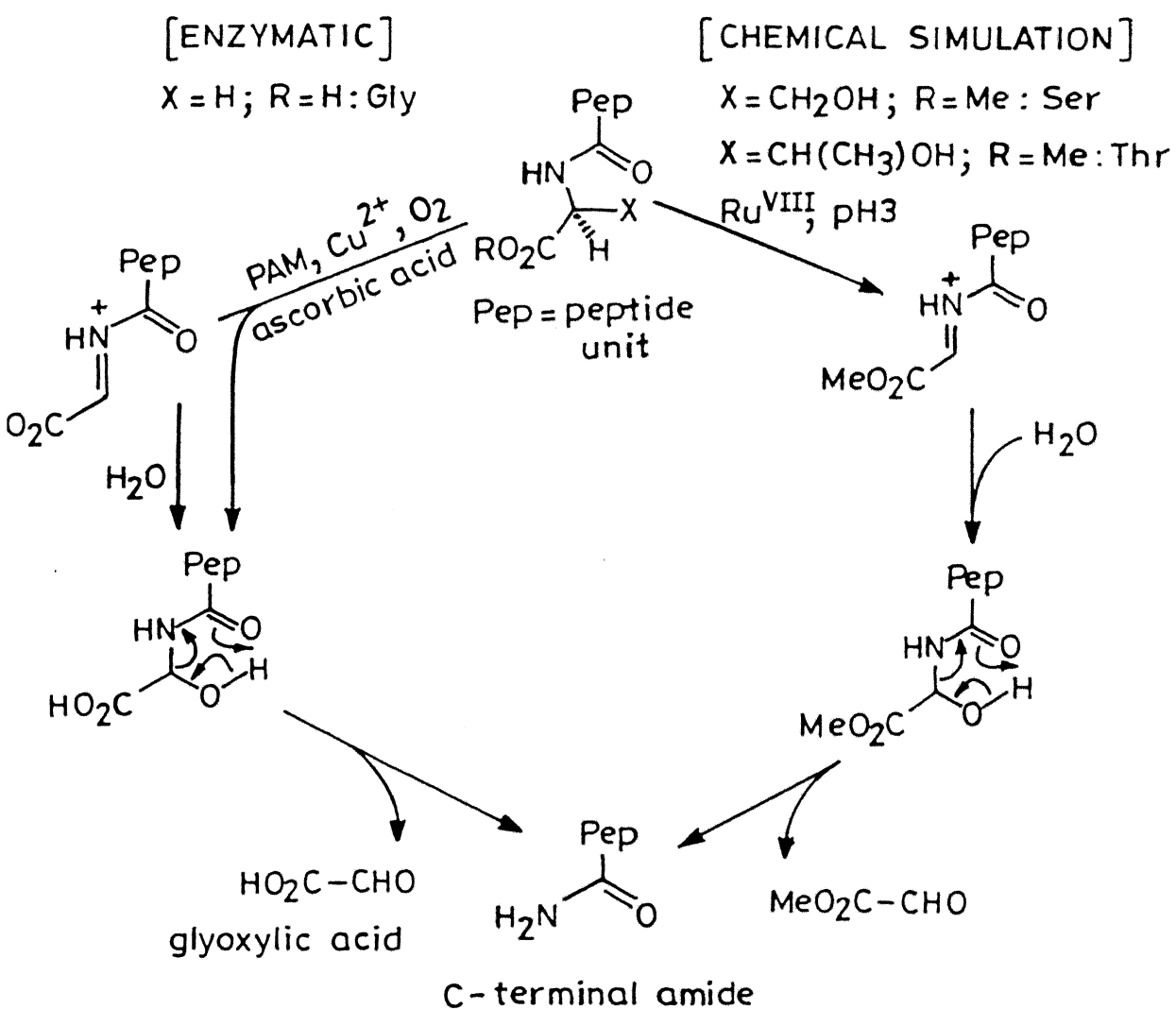
The successful chemical simulation of PAM action made it natural to explore the versatility of this novel finding in bringing about site selective scission in proteins. This obviously necessitated the synthesis of a large number of peptides wherein the key Ser/Thr residues are placed in all possible environments. In this effort, a total of 70 peptides were prepared and fully characterized. A study of these substrates from vantage of C^α -C side chain bond scission mediated by *in situ* generated Ru(VIII) species brought to light many latent facets having implications not only relating to the PAM terminal amidation, but also, to a powerful methodology for protein backbone modification, other coded amino acids that can function as the chemical equivalents to the Gly residue in terminal amidation and to the delineation of extremely subtle aspects that control the overall course of the oxidation.

The protocol associated with this study necessitated the synthesis of the peptide (*vide supra*), their reaction with *in situ* generated Ru(VIII)

Scheme C.2 Oxidative Scission of Ser/Thr extended precursors with Ru VIII :
Generation of C-terminal amides.



heme C.3 C-Terminal α -amidation of peptides: Chemical vs Enzymat



the extension of the concepts developed here to the synthesis of novel retropeptido-mimetic oxalamido unit containing peptides, crafted from vantage of synthesis of modular units having a bearing on protein function, protein design and having applications pertaining to, inter or intra-strand cross linking, design of inhibitors, crafting of transition state mimics and the preparation of hormone antagonists. These endeavours are taken up in their sequential order below.

L-Serine was N,C-protected to afford Bz-Ser-OMe (1) and o-NO₂-Bz-Ser-OMe (3) by esterification with methanolic HCl followed by, either benzylation in aq. bicarbonate or o-NO₂-benzylation in CH₂Cl₂-Et₃N (Chart C.1).

Bz-Ser-OMe (1): (83%)

mp. : 86°C (lit.¹⁰⁷ mp. 86°C).

ir : ν_{\max} (KBr)cm⁻¹: 3430 (OH), 3300 (NH), 1740 (ester), 1620 (amide I), 1530 (amide II).

nmr : δ (CDCl₃): 3.15 (1H, br, Ser OH), 3.71 (3H, s, COOCH₃), 3.95 (2H, dd, Ser C ^{β} H₂), 4.80 (1H, m, Ser C ^{α} H), 7.11-8.05 (6H, m, NH, aromatic protons).

o-NO₂-Bz-Ser-OMe (3): (50%)

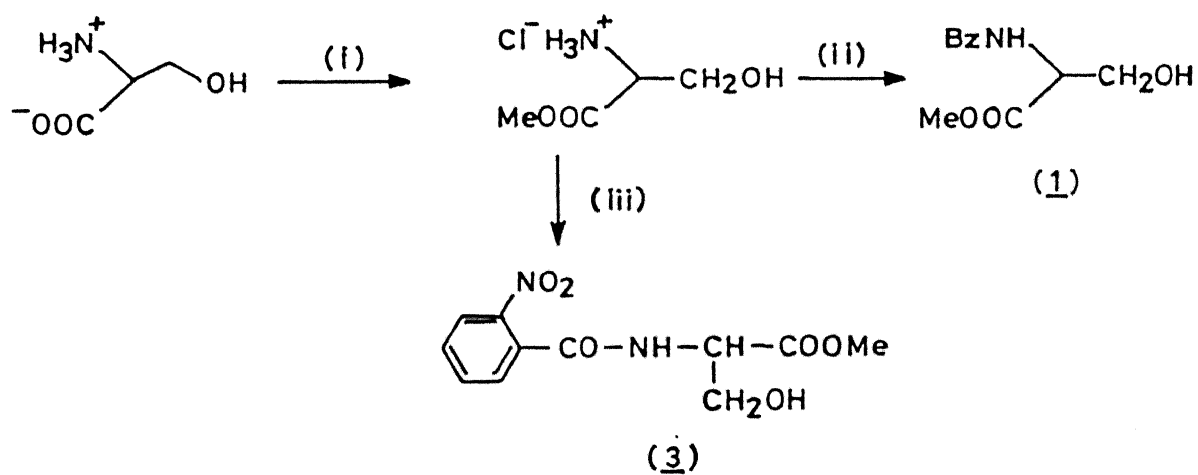
mp. : 92°C

ir : ν_{\max} (KBr)cm⁻¹: 3568 (OH), 3283 (NH), 1743 (ester), 1634 (amide I), 1611, 1573 (amide II), 1522 (NO₂), 1356 (NO₂).

nmr : δ (CDCl₃): 3.84 (3H, s, COOCH₃), 4.09 (2H, m, Ser C ^{β} H₂), 4.81 (1H, m, Ser C ^{α} H), 6.79 (1H, br, NH), 7.34-8.28 (4H, m, aromatic protons).

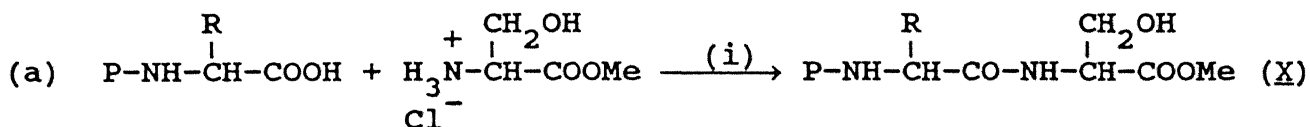
Thirteen dipeptides having C-terminal Ser residues were prepared by condensation of the appropriate N-protected coded amino acids with *in situ*

CHART C.1



- (i) $\text{MeOH/HCl}/0^\circ$;
(ii) BzCl/Aq. NaHCO_3 ;
(iii) o-nitrobenzoyl chloride/ $\text{NEt}_3/\text{CH}_2\text{Cl}_2$

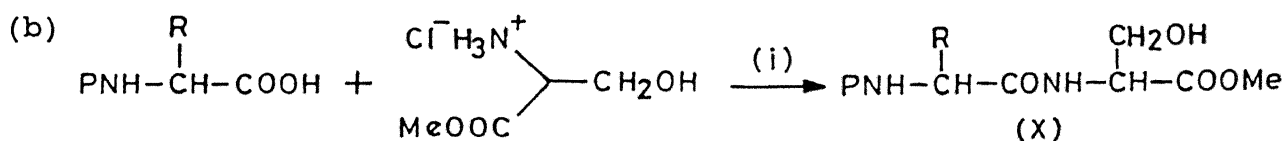
CHART C.2



P	R	(X)
Z	H	Z-Gly-Ser-OMe (<u>5</u>)
Bz	H	Bz-Gly-Ser-OMe (<u>7</u>)
Bz	CH ₃	Bz-Ala-Ser-OMe (<u>9</u>)
Bz	CH ₂ CH(CH ₃) ₂	Bz-Leu-Ser-OMe (<u>11</u>)
Z	CH ₂ CH(CH ₃) ₂	Z-Leu-Ser-OMe (<u>11a</u>)
Bz	CH ₂ Ph	Bz-Phe-Ser-OMe (<u>13</u>)
Bz	CH ₂ COOMe	Bz-Asp(β-OMe)-Ser-OMe (<u>15</u>)
Boc	CH ₂ COOCH ₂ Ph	Boc-Asp(β-OBzl)-Ser-OMe (<u>17</u>)
Bz	CH ₂ CH ₂ COOMe	Bz-Glu(γ-OMe)-Ser-OMe (<u>19</u>)
Z	CH ₂ CH ₂ SMe	Z-Met-Ser-OMe (<u>25</u>)
Bz	CH(CH ₃) ₂	Bz-Val-Ser-OMe (<u>27</u>)
Bz	[Pro]	Bz-Pro-Ser-OMe (<u>29</u>)
Boc	(CH ₂) ₃ -N- $\begin{matrix} \text{N-NO}_2 \\ \text{NH}_2 \end{matrix}$	Boc-Arg(N ^G NO ₂)-Ser-OMe (<u>31</u>)

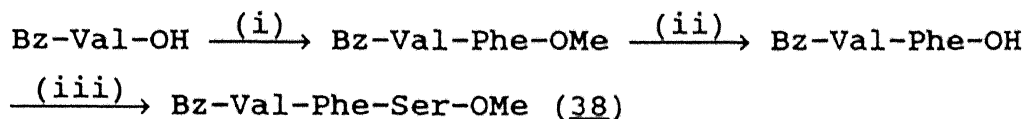
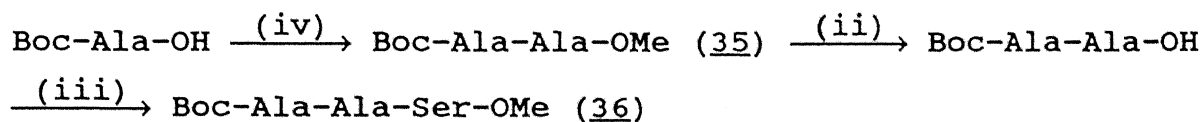
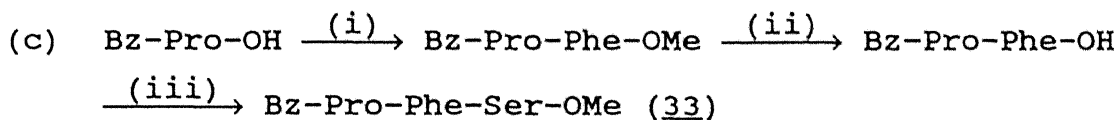
(i) HOBT/DCC/CH₂Cl₂-DMF/NEt₃

CHART C.2 (CONTINUED)



P	R	(<u>X</u>)
Z	CH ₂ CONH ₂	Z-Asn-Ser-OMe (<u>21</u>)
Z	CH ₂ CH ₂ CONH ₂	Z-Gln-Ser-OMe (<u>23</u>)

(i) (PhO)₂P(O)N₃/NEt₃/DMF



(i) H-Phe-OMe.HCl/NEt₃/DCC/HOBt/CH₂Cl₂-DMF;

(ii) Aq. NaOH-MeOH;

(iii) H-Ser-OMe.HCl/NEt₃/DCC/HOBt/CH₂Cl₂-DMF;

(iv) H-Ala-OMe.HCl/NEt₃/DCC/HOBt/CH₂Cl₂-DMF

Z-Gly-Ser-OMe (5): (94%)

mp. : 94-95°C (lit.¹⁰⁹ mp. 96°C)

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3395 (OH), 3310 (NH), 1733 (ester), 1718, 1688 (amide I), 1657 (amide I), 1540 (amide II), 1513 (amide II).

nmr : $\delta(\text{CDCl}_3)$: 3.73 (5H, s + m, COOCH_3 + Ser C^βH_2), 3.89 (2H, d, $J=5$ Hz, Gly CH_2), 4.60 (1H, m, Ser C^αH), 5.09 (2H, s, Z CH_2), 6.00 (1H, t, exchangeable with D_2O , Gly NH), 7.32 (6H, s, Ser NH + aromatic protons).

anal: Found: C, 54.33; H, 5.65; N, 8.87 %

Calc. for $\text{C}_{14}\text{H}_{18}\text{N}_2\text{O}_6$: C, 54.19; H, 5.81; N, 9.03 %

Bz-Gly-Ser-OMe (7): (70%)

mp. : 82-84°C

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3360 (OH), 3285 (NH), 1730 (ester), 1655 (amide I), 1630 (amide I), 1555 (amide II).

nmr : $\delta[\text{CDCl}_3 + (\text{CD}_3)_2\text{SO}]$: 3.80 (3H, s, COOCH_3), 3.91 (2H, m, Ser C^βH_2), 4.14 (2H, d, $J=5$ Hz, Gly CH_2), 4.60 (1H, m, Ser C^αH), 7.20-8.25 (7H, m, Ser NH + Gly NH + aromatic protons).

ms : m/z : 281 (MH)⁺.

anal: found: C, 55.44; H, 5.98; N, 10.09 %

Calc. for $\text{C}_{13}\text{H}_{16}\text{N}_2\text{O}_5$: C, 55.71; H, 5.71; N, 10.00 %

$[\alpha]_{\text{D}}^{30}$: -2.3 (c, 3.3, MeOH).

Bz-Ala-Ser-OMe (9): (65%)

mp. : 135-136°C (lit.¹¹⁵)

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3455 (OH), 3325 (NH), 1740 (ester), 1655 (amide I), 1625 (amide I), 1600, 1570 (amide II), 1530

nmr : δ [CDCl₃ + (CD₃)₂SO]: 1.47 (3H, d, J=7.5 Hz, Ala CH₃), 3.75 (3H, s, COOCH₃), 3.87 (2H, m, Ser C ^{β} H₂), 4.39-4.95 (2H, m, Ala C ^{α} H + Ser C ^{α} H), 7.20-8.00 (7H, m, Ala NH + Ser NH + aromatic protons).

anal: found: C, 57.42; H, 6.38; N, 9.71 %

Calc. for C₁₄H₁₈N₂O₅: C, 57.14; H, 6.12; N, 9.52 %

$[\alpha]_D^{30}$: +10.8 (c, 0.4, MeOH).

Bz-Leu-Ser-OMe (11): (70%)

mp. : 95-97°C

ir : ν_{\max} (KBr)cm⁻¹: 3284 (NH), 1750 (ester), 1638 (amide I) 1544 (amide II).

anal: Found: C, 60.36; H, 7.42; N, 8.42 %

Calc. for C₁₇H₂₄N₂O₅: C, 60.71; H, 7.14; N, 8.33 %

$[\alpha]_D^{30}$: +24.1 (c, 3.3, MeOH).

Z-Leu-Ser-OMe (11a): (89%)

mp. : 114-115°C (lit.¹⁵⁹ mp. 116°C)

Bz-Phe-Ser-OMe (13): (63%)

mp. : 105-106°C

ir : ν_{\max} (KBr)cm⁻¹: 3330 (OH), 3280 (NH), 1740 (ester), 1725, 1635 (amide I), 1575 (amide II), 1545 (amide II), 1535 (amide II).

ms : m/z: 370 (M)⁺.

anal: Found: C, 65.07; H, 6.23; N, 7.73 %

Calc. for C₂₀H₂₂N₂O₅: C, 64.86; H, 5.94; N, 7.57 %

$[\alpha]_D^{30}$: +2.1 (c, 3.3, MeOH).

Bz-Asp(β -OMe)-Ser-OMe (15): (78%)

mp. : 135-136°C

1652 (amide I), 1562 (amide II), 1542 (amide II).

nmr : $\delta(\text{CDCl}_3)$: 2.94 (2H, dd, $J=5.5$ Hz, 1Hz, Asp C^βH_2), 3.72, 3.75 (3H, 3H, s, s, $\text{COOCH}_3 \times 2$), 3.91 (2H, d, $J=3$ Hz, Ser C^βH_2), 4.56 (1H, m, Ser C^αH), 5.00 (1H, m, Asp C^αH), 7.28-7.84 (7H, m, Asp NH + Ser NH + aromatic protons).

ms : m/z : 353 (MH)⁺.

anal: Found: C, 54.86; H, 5.90; N, 8.15 %

Calc. for $\text{C}_{16}\text{H}_{20}\text{N}_2\text{O}_7$: C, 54.54; H, 5.68; N, 7.95 %

Boc-Asp(β -OBzl)-Ser-OMe (17): (58%)

mp. : syrup

ir : $\nu_{\text{max}}(\text{KBr})\text{cm}^{-1}$: 3370 (br, NH, OH), 1740 (ester), 1670 (amide I), 1530 (amide II).

nmr : $\delta(60 \text{ MHz}, \text{CDCl}_3)$: 1.43 (9H, s, Boc $\text{CH}_3 \times 3$), 2.83 (2H, d, $J=5.5$ Hz, Asp C^βH_2), 3.70 (3H, s, COOCH_3), 3.80 (2H, br, Ser C^βH_2) 4.53 (2H, m, Asp C^αH + Ser C^αH), 5.06 (2H, s, Bzl CH_2), 6.03 (1H, d, $J=8.7$ Hz, Asp NH), 7.23 (5H, s, aromatic protons), 7.46 (1H, d, $J=7.25$ Hz, Ser NH).

anal : Found: C, 56.47; H, 6.38; N, 6.87 %

Calc. for $\text{C}_{20}\text{H}_{28}\text{N}_2\text{O}_8$: C, 56.60; H, 6.60; N, 6.60 %

$[\alpha]_{\text{D}}^{30}$: +25.53 (c, 0.32, CHCl_3).

Bz-Glu(γ -OMe)-Ser-OMe (19): (65%)

mp. : 134-136°C

ir : $\nu_{\text{max}}(\text{KBr})\text{cm}^{-1}$: 3273 (br, NH, OH), 1734 (ester), 1707, 1655 (amide I), 1626 (amide I), 1576 (amide II), 1532 (amide II).

nmr : $\delta(\text{CDCl}_3)$: 2.09-2.71 (4H, m, Glu C^βH_2 + Glu $\text{C}^\gamma\text{H}_2$), 3.68, 3.76 (3H, 3H, s, s, $\text{COOCH}_3 \times 2$), 4.00 (2H, m, Ser C^βH_2), 4.68 (2H, m, Glu C^αH + Ser C^αH), 7.34-8.00 (7H, m, Glu NH + Ser NH + aromatic protons).

anal: Found: C, 55.64; H, 6.36; N, 7.33 %

Calc. for $C_{17}H_{22}N_2O_7$: C, 55.74; H, 6.01; N, 7.65 %

Z-Met-Ser-OMe (25): (90%)

mp. : 143-144°C

ir : ν_{\max} (KBr) cm^{-1} : 3535 (OH), 3300 (NH), 1725 (ester), 1682 (amide I), 1645 (amide I), 1555 (amide II), 1540 (amide II).

nmr : δ (CDCl_3): 2.09 (5H, s + m, Met C^βH_2 + Met S- CH_3), 2.56 (2H, t, Met $\text{C}^\gamma\text{H}_2$), 3.75 (3H, s, COOCH_3), 3.87 (2H, brd, Ser C^βH_2), 4.15-4.75 (2H, m, Met C^αH + Ser C^αH), 5.06 (2H, s, Z CH_2), 5.62 (1H, d, $J=7.5$ Hz, Met NH), 6.90-7.53 (6H, s + m, Ser NH + aromatic protons).

anal: Found: C, 53.40; H, 6.38; N, 7.26 %

Calc. for $C_{17}H_{24}N_2O_6S$: C, 53.12; H, 6.25; N, 7.29 %

$[\alpha]_D^{30}$: +20.94 (c, 0.42, CHCl_3).

Bz-Val-Ser-OMe (27): (78%)

mp. : 169-170°C

ir : ν_{\max} (KBr) cm^{-1} : 3340 (OH), 3290 (NH), 1750 (ester), 1623 (amide I), 1570 (amide II).

nmr : δ (CDCl_3): 1.06 (6H, d, $J=5.0$ Hz, Val $\text{CH}_3 \times 2$), 2.18 (1H, m, Val C^βH), 3.65-4.10 (5H, s + m, COOCH_3 + Ser C^βH_2), 4.62 (2H, m, Val C^αH + Ser C^αH), 6.93-8.00 (7H, m, Val NH + Ser NH + aromatic protons).

anal: Found: C, 59.86; H, 6.47; N, 8.58 %

Calc. for $C_{16}H_{22}N_2O_5$: C, 59.63; H, 6.83; N, 8.70 %

$[\alpha]_D^{30}$: +15.44 (c, 1.58, CHCl_3).

Bz-Pro-Ser-OMe (29): (40%)

mp. : 71-72°C

ir : ν_{\max} (KBr) cm^{-1} : 3460 (OH), 3390 (NH), 3320 (NH), 1743 (ester), 1642 (amide I), 1613 (amide I), 1570 (amide II),

nmr : $\delta(\text{CDCl}_3)$: 2.18 (4H, m, Pro C^βH_2 + Pro $\text{C}^\gamma\text{H}_2$), 3.46-4.12 (7H, s + m, COOCH_3 + Pro $\text{C}^\delta\text{H}_2$ + Ser C^βH_2), 4.62 (2H, m, Pro C^αH + Ser C^αH), 7.03-7.81 (6H, m, Ser NH + aromatic protons).

anal: Found: C, 59.68; H, 6.52; N, 8.57 %

Calc. for $\text{C}_{16}\text{H}_{20}\text{N}_2\text{O}_5$: C, 60.00; H, 6.25; N, 8.75 %

$[\alpha]_D^{30}$: -26.50 (c, 0.8, CHCl_3).

Boc-Arg(N^GNO_2)-Ser-OMe (31): (52%)

mp. : 74°C

ir : $\nu_{\text{max}}(\text{KBr})\text{cm}^{-1}$: 3318 (br, OH, NH), 1744 (ester), 1661 (amide I), 1600, 1532 (br, amide II, NO_2), 1355 (NO_2).

nmr : $\delta[\text{CDCl}_3 + (\text{CD}_3)_2\text{SO}]$: 1.44 (9H, s, Boc $\text{CH}_3 \times 3$), 1.72 (4H, m, Arg C^βH_2 + Arg $\text{C}^\gamma\text{H}_2$), 3.34 (2H, m, Arg $\text{C}^\delta\text{H}_2$), 3.72-4.00 (5H, s + m, COOCH_3 + Ser C^βH_2), 4.12 (1H, m, Arg C^αH), 4.52 (1H, m, Ser C^αH), 6.31 (1H, d, $J=7.5$ Hz, Arg NH), 7.56-8.18 (4H, m, Ser NH + Guanidinium $\text{NH} \times 3$).

ms : m/z : 421 (MH) $^+$.

anal: Found: C, 43.25; H, 6.80; N, 19.64 %

Calc. for $\text{C}_{15}\text{H}_{28}\text{N}_6\text{O}_8$: C, 42.85; H, 6.66; N, 20.00 %

Z-Asn-Ser-OMe (21) and Z-Gln-Ser-OMe (23) were prepared from the appropriate precursors by using diphenylphosphoryl azide as the coupling agent (Chart C.2.b).

Z-Asn-Ser-OMe (21): (65%)

mp. : $194-195^\circ\text{C}$ (lit. 119 mp. $197-199^\circ\text{C}$)

ir : $\nu_{\text{max}}(\text{KBr})\text{cm}^{-1}$: 3421 (OH), 3298 (NH), 1731 (ester), 1686 (amide I), 1653 (amide I), 1609, 1539 (amide II).

nmr : $\delta[\text{CDCl}_3 + (\text{CD}_3)_2\text{SO}]$: 2.50 (2H, d, $J=5$ Hz, Asn C^βH_2), 3.68 (5H, s+m, COOCH_3 + Ser C^βH_2), 4.45 (2H, m, Asn C^αH + Ser

brs, Asn CONH₂ + aromatic protons), 8.09 (1H, d, J=8.75 Hz, Ser NH).

anal: Found: C, 52.43; H, 5.63; N, 11.62 %

Calc. for C₁₆H₂₁N₃O₇: C, 52.32; H, 5.72; N, 11.44 %

γ -Gln-Ser-OMe (23): (63%)

mp. : 156-160°C (lit.¹²¹ mp. 156-160°C)

ir : ν_{\max} (KBr)cm⁻¹: 3403 (OH), 3312 (NH), 1747 (ester), 1642 (amide I), 1535 (amide II).

nmr : δ [CDCl₃ + (CD₃)₂SO]: 1.68-2.40 (4H, brm, Gln C ^{β} H₂ + Gln C ^{γ} H₂), 3.68 (5H, brs, COOCH₃ + Ser C ^{β} H₂), 4.00-4.59 (2H, m, Gln C ^{α} H + Ser C ^{α} H), 5.06 (2H, s, Z CH₂), 6.50 (1H, br, Gln NH), 6.84-7.56 (7H, s + br, Gln CONH₂ + aromatic protons), 8.03 (1H, br, Ser NH).

anal: Found: C, 53.93; H, 6.18; N, 11.44 %

Calc. for C₁₇H₂₃N₃O₇: C, 53.54; H, 6.04; N, 11.02 %

The tripeptides Bz-Pro-Phe-Ser-OMe (33), Boc-Ala-Ala-Ser-OMe (36) and -Val-Phe-Ser-OMe (38) were prepared as shown in Chart C.2.c.

γ -Pro-Phe-Ser-OMe (33): (68%)

mp. : 182-184°C

ir : ν_{\max} (KBr)cm⁻¹: 3400 (OH), 3340 (NH), 1742 (ester), 1660 (amide I), 1600, 1570 (amide II), 1535 (amide II).

nmr : δ (CDCl₃): 1.96 (4H, m, Pro C ^{β} H₂ + Pro C ^{γ} H₂), 3.15-3.93 (9H, s + m, COOCH₃ + Pro C ^{δ} H₂ + Phe C ^{β} H₂ + Ser C ^{β} H₂), 4.50 (3H, m, Pro C ^{α} H + Phe C ^{α} H + Ser C ^{α} H), 6.90 (1H, d, J=7.5 Hz, Phe NH), 7.15-7.59 (11H, s + m, Ser NH + aromatic protons).

anal: Found: C, 64.21; H, 6.32; N, 8.72 %

Calc. for C₂₅H₂₉N₃O₆: C, 64.24; H, 6.21; N, 8.99 %

Boc-Ala-Ala-OMe (35): (82%)

mp. : 98-99°C (lit.¹²⁷)

ir : ν_{\max} (KBr) cm^{-1} : 3260 (br, NH), 1725 (ester), 1640 (br, amide I), 1525 (br, amide II).

Boc-Ala-Ala-Ser-OMe (36): (68%)

mp. : 156-158°C

nmr : δ (CDCl_3): 1.43 (15H, s + m, Boc $\text{CH}_3 \times 3$ + Ala $\text{CH}_3 \times 2$), 3.81 (3H, s, COOCH_3), 3.96 (2H, m, Ser $\text{C}^\beta \text{H}_2$), 4.18 (1H, m, Ser $\text{C}^\alpha \text{H}$), 4.62 (2H, m, Ala $\text{C}^\alpha \text{H} \times 2$), 5.28 (1H, d, $J=7.5$ Hz, Ala NH(Boc)), 7.06 (1H, d, $J=7.5$ Hz, NH), 7.46 (1H, d, $J=7.5$ Hz, NH).

anal: Found: C, 49.58; H, 7.33; N, 11.52 %

Calc. for $\text{C}_{15}\text{H}_{27}\text{N}_3\text{O}_7$: C, 49.86; H, 7.48; N, 11.63 %

$[\alpha]_D^{30}$: -24.00 (c, 0.5, CHCl_3).

Bz-Val-Phe-Ser-OMe (38) : (87%)

mp. : 165-167°C

ir : ν_{\max} (KBr) cm^{-1} : 3338 (NH), 3298 (NH), 1743 (ester), 1630 (amide I), 1580, 1538 (amide II).

anal: Found: C, 64.34; H, 6.76; N, 8.79 %

Calc. for $\text{C}_{25}\text{H}_{31}\text{N}_3\text{O}_6$: C, 63.96; H, 6.61; N, 8.95 %

$[\alpha]_D^{30}$: -13.9 (c, 3.3, MeOH).

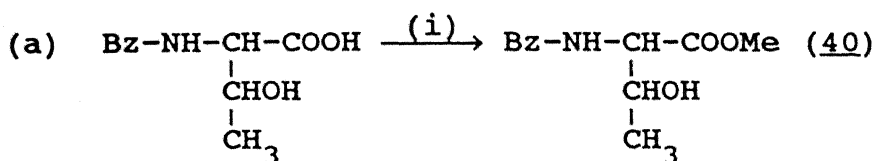
Bz-Thr-OMe (40) was prepared by diazomethane esterification (Chart C.3.a).

Bz-Thr-OMe (40): (79%)

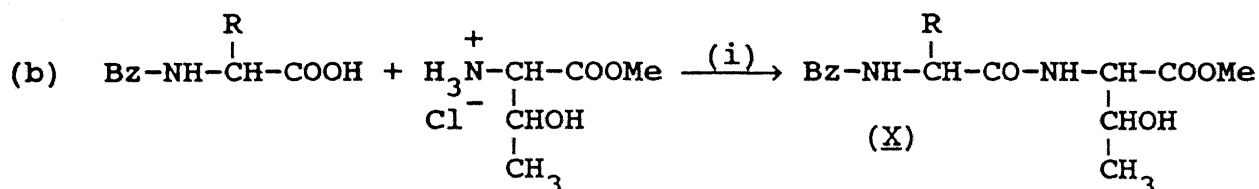
mp. : 91-92°C (lit.¹²⁹ mp. 96°C)

ir : ν_{\max} (KBr) cm^{-1} : 3410 (OH), 3345 (NH), 1730 (ester), 1630 (amide I), 1510 (amide II).

CHART C.3

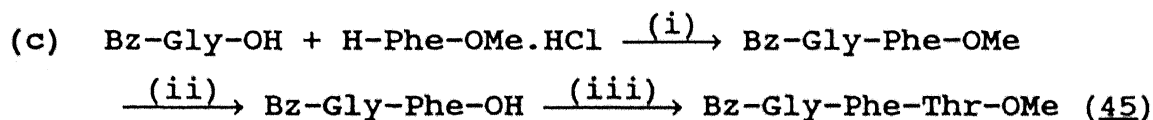


(i) $\text{CH}_2\text{N}_2/\text{Et}_2\text{O}$



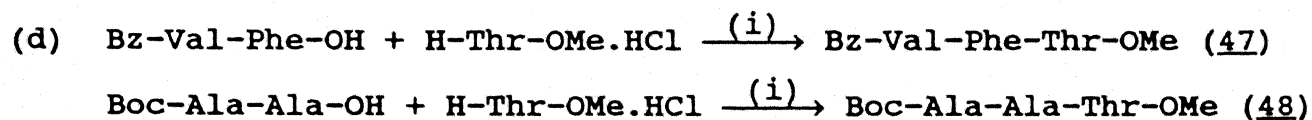
R	(X)
H	Bz-Gly-Thr-OMe (41)
CH ₃	Bz-Ala-Thr-OMe (42)
CH ₂ CH(CH ₃) ₂	Bz-Leu-Thr-OMe (43)
CH ₂ Ph	Bz-Phe-Thr-OMe (44)

(i) HOBT/DCC/CH₂Cl₂-DMF/NEt₃



(i) HOBT/DCC/CH₂Cl₂/NEt₃; (ii) 2N NaOH-MeOH/0°/rt/4 h;

(iii) H-Thr-OMe.HCl/NEt₃/DCC/HOBT/CH₂Cl₂



(i) HOBT/DCC/CH₂Cl₂-DMF/NEt₃

Bz-Phe-Thr-OMe (44) were prepared by condensation of *in situ* generated Thr-OMe with appropriate Bz-N-protected amino acids using HOBt/DCC in CH_2Cl_2 -DMF (Chart C.3.b).

Bz-Gly-Thr-OMe (41): (90%)

mp. : 138-140°C

ir : ν_{max} (KBr) cm^{-1} : 3485 (OH), 3370 (NH), 3315 (NH), 1723 (ester), 1671 (amide I), 1647 (amide I), 1580 (amide II), 1549 (amide II).

nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 1.15 (3H, d, $J=6.5$ Hz, Thr CH_3), 3.71 (3H, s, COOCH_3), 4.12 (2H, d, $J=5.0$ Hz, Gly CH_2), 4.25 (1H, m, Thr C^βH), 4.46 (1H, dd, $J=8.75$ Hz, 2.5 Hz, Thr C^αH), 7.28-8.06 (6H, m, Gly NH + aromatic protons), 8.37 (1H, m, Thr NH).

anal: Found: C, 57.40; H, 6.26; N, 9.83 %

Calc. for $\text{C}_{14}\text{H}_{18}\text{N}_2\text{O}_5$: C, 57.14; H, 6.12; N, 9.52 %

$[\alpha]_{\text{D}}^{30}$: -6.21 (c, 3.3, MeOH).

Bz-Ala-Thr-OMe (42): (64%)

mp. : 68-70°C (lit.¹³⁰)

ir : ν_{max} (KBr) cm^{-1} : 3320 (NH), 1740 (ester), 1660 (amide I), 1530 (amide II).

nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 1.15 (3H, d, $J=6.5$ Hz, Thr CH_3), 1.53 (3H, d, $J=6.5$ Hz, Ala CH_3), 3.78 (3H, s, COOCH_3), 4.28 (1H, m, Thr C^βH), 4.56 (1H, dd, $J=8.75$ Hz, 2.5 Hz, Thr C^αH), 4.88 (1H, m, Ala C^αH), 7.19-8.00 (7H, m, Ala NH + Thr NH + aromatic protons).

ms : m/z : 309 (MH)⁺.

anal: Found: C, 57.73; H, 6.82; N, 8.49 %

Calc. for $\text{C}_{15}\text{H}_{20}\text{N}_2\text{O}_5$: C, 58.44; H, 6.49; N, 9.09 %

$[\alpha]_D^{30}$: +6.1 (c, 3.3, CHCl_3).

Bz-Leu-Thr-OMe (43): (65%)

mp. : 113-114°C

ir : ν_{max} (KBr) cm^{-1} : 3300 (NH), 1747 (ester), 1670 (amide I),
1639 (amide I), 1537 (amide II).

nmr : δ (CDCl_3): 0.94 (6H, brs, Leu $\text{CH}_3 \times 2$), 1.20 (3H, d, $J=6.5$
Hz, Thr CH_3), 1.73 (3H, br, Leu C^βH_2 + Leu C^γH), 3.76 (3H,
s, COOCH_3), 4.00 (1H, br, Thr OH), 4.31 (1H, br, Thr C^βH),
4.56 (1H, m, Thr C^αH), 4.85 (1H, m, Leu C^αH), 7.05-8.14
(7H, m, Leu NH + Thr NH + aromatic protons).

ms : m/z: 351 (MH^+).

anal: Found: C, 61.23; H, 7.18; N, 8.22 %

Calc. for $\text{C}_{18}\text{H}_{26}\text{N}_2\text{O}_5$: C, 61.71; H, 7.43; N, 8.00 %

$[\alpha]_D^{30}$: -5.4 (c, 3.3, MeOH).

Bz-Phe-Thr-OMe (44): (63%)

mp. : 145-146°C

ir : ν_{max} (KBr) cm^{-1} : 3475 (OH), 3310 (NH), 3270 (NH), 1720
(ester), 1671 (amide I), 1641 (amide I), 1541 (amide II).

ms : m/z: 384 (M^+).

anal: Found: C, 65.69; H, 6.37; N, 7.18 %

Calc. for $\text{C}_{21}\text{H}_{24}\text{N}_2\text{O}_5$: C, 65.62; H, 6.25; N, 7.29 %

The tripeptides Bz-Gly-Phe-Thr-OMe (45), Bz-Val-Phe-Thr-OMe (47) and
Boc-Ala-Ala-Thr-OMe (48) were prepared as shown in Chart C.3.c and Chart
C.3.d.

Bz-Gly-Phe-Thr-OMe (45): (79%)

mp. : 157-159°C

ir : ν_{max} (KBr) cm^{-1} : 3320 (NH), 1750 (ester), 1660. (amide I),

nmr : δ [$\text{CDCl}_3 + (\text{CD}_3)_2\text{SO}$]: 1.14 (3H, d, $J=6.5$ Hz, Thr CH_3), 3.12 (2H, m, Phe C^βH_2), 3.70 (3H, s, COOCH_3), 3.96 (2H, dd, $J=5.0$ Hz, 1.0 Hz, Gly CH_2), 4.19-4.60 (2H, m, Thr $\text{C}^\beta\text{H} + \text{Thr C}^\alpha\text{H}$), 4.75 (1H, m, Phe C^αH), 7.00-8.00 (12H, m, Phe NH + Thr NH + aromatic protons), 8.15 (1H, t, Gly NH).

ms : m/z : 441 (M)⁺.

anal: Found: C, 62.38; H, 6.29; N, 9.17 %

Calc. for $\text{C}_{23}\text{H}_{27}\text{N}_3\text{O}_6$: C, 62.58; H, 6.12; N, 9.52 %

Bz-Val-Phe-Thr-OMe (47): (52%)

mp. : 205-207°C

ir : ν_{max} (KBr) cm^{-1} : 3495 (OH), 3340 (NH), 3320 (NH), 3280 (NH), 1722 (ester), 1630 (amide I), 1580, 1540 (amide II).

nmr : δ [$\text{CDCl}_3 + (\text{CD}_3)_2\text{SO}$]: 0.75-1.34 (9H, m, Thr $\text{CH}_3 + \text{Val CH}_3 \times 2$), 2.14 (1H, m, Val C^βH), 3.15 (2H, t, Phe C^βH_2), 3.75 (3H, s, COOCH_3), 4.18-5.15 (4H, m, Val $\text{C}^\alpha\text{H} + \text{Phe C}^\alpha\text{H} + \text{Thr C}^\alpha\text{H} + \text{Thr C}^\beta\text{H}$), 7.09-8.00 (13H, m, Val NH + Phe NH + Thr NH + aromatic protons).

anal: Found: C, 64.65; H, 6.67; N, 8.56 %

Calc. for $\text{C}_{26}\text{H}_{33}\text{N}_3\text{O}_6$: C, 64.60; H, 6.83; N, 8.69 %

$[\alpha]_D^{30}$: -20.9 (c, 2.3, MeOH).

Boc-Ala-Ala-Thr-OMe (48): (53%)

mp. : 155-156°C

ir : ν_{max} (KBr) cm^{-1} : 3390 (OH), 3310 (NH), 1740 (ester), 1695 (carbamate), 1638 (amide I), 1530 (amide II).

nmr : δ [$\text{CDCl}_3 + (\text{CD}_3)_2\text{SO}$]: 1.03-1.60 (15H, m, Thr $\text{CH}_3 + \text{Ala CH}_3 \times 2 + \text{Boc CH}_3 \times 3$), 3.81 (3H, s, COOCH_3), 4.06-4.81 (4H, m, Ala $\text{C}^\alpha\text{H} \times 2 + \text{Thr C}^\alpha\text{H} + \text{Thr C}^\beta\text{H}$), 5.65 (1H, d, $J=7.5$ Hz, Ala NH(Boc)), 7.46 (2H, m, Ala NH + Thr NH).

Calc. for $C_{16}H_{29}N_3O_7$: C, 51.20; H, 7.73; N, 11.20 %
[α]_D³⁰: -53.9 (c, 3.3, MeOH).

It may be noted that the peptides thus far prepared had Ser/Thr-OMe as C-terminal residues. A similar series of compounds were prepared wherein Ser/Thr residue was placed at the N-terminal site.

The condensation of Z-Ser with a range of *in situ* generated coded amino acid esters by the HOBt/DCC procedure in CH_2Cl_2 -DMF afforded nine dipeptides having Z-protected serine residue at the N-terminal location (Chart C.4.a).

Z-Ser-Gly-OMe (49): (78%)

mp. : 98-99°C (lit.¹³³ mp. 105-106°C)

ir : ν_{\max} (KBr) cm^{-1} : 3310 (NH), 1753 (ester), 1682 (amide I),
1648 (amide I), 1529 (amide II).

nmr : δ ($CDCl_3$): 3.71 (5H, s + m, $COOCH_3$ + Ser $C^{\beta}H_2$), 4.00 (2H, d, $J=6.25$ Hz, Gly CH_2), 4.26 (1H, m, Ser $C^{\alpha}H$), 5.12 (2H, s, Z CH_2), 5.96 (1H, d, $J=7.5$ Hz, Ser NH), 6.96-7.46 (6H, s + m, Gly NH + aromatic protons).

anal: Found: C, 54.23; H, 5.55; N, 9.25 %

Calc. for $C_{14}H_{18}N_2O_6$: C, 54.19; H, 5.81; N, 9.03 %
[α]_D²⁵: -8.1 (c, 3.3, $CHCl_3$).

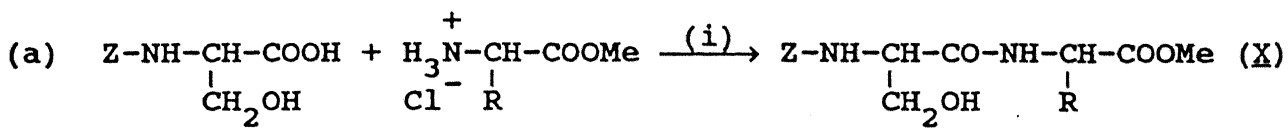
Z-Ser-Ala-OMe (51): (78%)

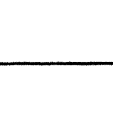
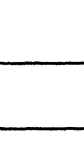
mp. : 104-106°C (lit.¹³⁴ mp. 113-114°C)

ir : ν_{\max} (KBr) cm^{-1} : 3314 (NH), 1762 (ester), 1694 (carbamate),
1657 (amide I), 1539 (amide II).

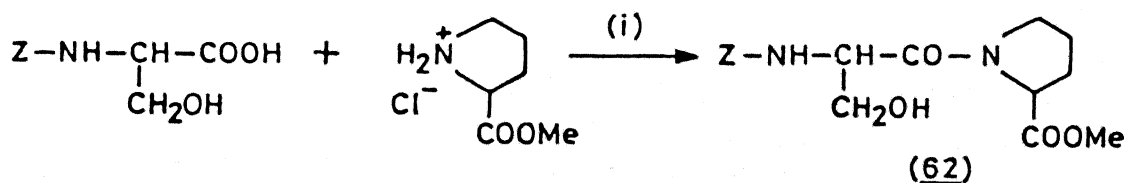
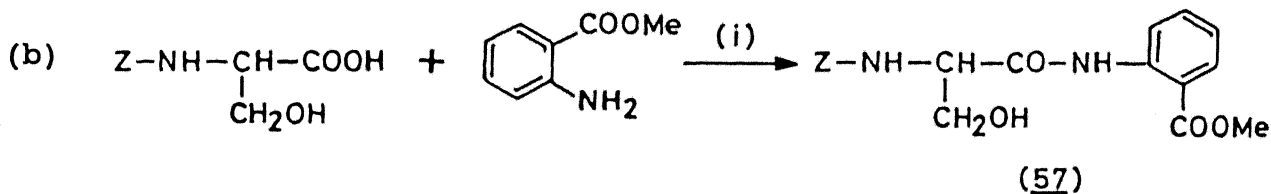
nmr : δ ($CDCl_3$): 1.33 (3H, d, $J=7.5$ Hz, Ala CH_3), 3.71 (5H, s+m, $COOCH_3$ + Ser $C^{\beta}H_2$), 4.15-4.75 (2H, m, Ala $C^{\alpha}H$ + Ser $C^{\alpha}H$), 5.09 (2H, s, Z CH_2), 6.03 (1H, d, $J=7.5$ Hz, Ser NH), 7.36 (6H, s+m, Ala NH + aromatic protons).

CHART C.4



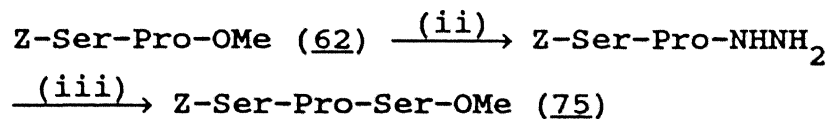
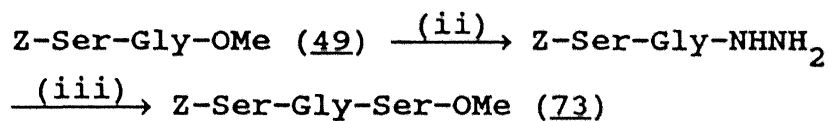
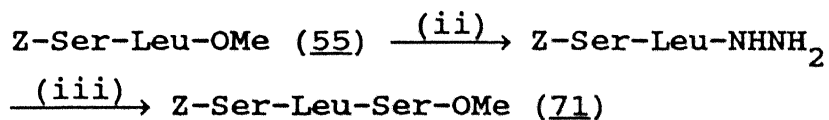
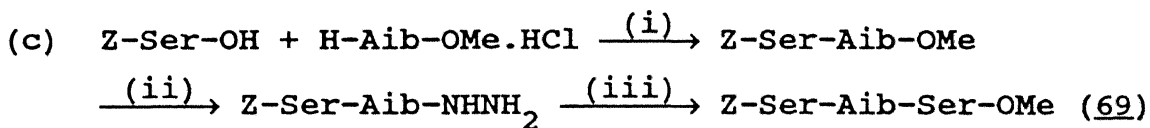
R	(X)
H	Z-Ser-Gly-OMe (49)
CH ₃	Z-Ser-Ala-OMe (51)
CH ₂ Ph	Z-Ser-Phe-OMe (53)
CH ₂ CH(CH ₃) ₂	Z-Ser-Leu-OMe (55)
CH ₂ - 	Z-Ser-Tyr-OMe (59)
CH ₂ - 	Z-Ser-Trp-OMe (61)
CH ₂ COOMe	Z-Ser-Asp(β-OMe)-OMe (64)
CH ₂ OH	Z-Ser-Ser-OMe (66)
CH ₂ CH ₂ SCH ₃	Z-Ser-Met-OMe (67)

(i) HOBT/DCC/CH₂Cl₂-DMF/NEt₃



(i) HOBT/DCC/CH₂Cl₂-DMF/NEt₃

CHART C.4 (CONTINUED)



(i) HOBT/DCC/ CH_2Cl_2 -DMF/ NEt_3 ;

(ii) $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$ /EtOH/rt/24 h;

(iii) Aq. AcOH/6N HCl/ $\text{NaNO}_2/0^\circ$; H-Ser-OMe.HCl/ $\text{NEt}_3/\text{CH}_2\text{Cl}_2/0^\circ$

anal: Found: C, 55.43; H, 6.37; N, 8.59 %

Calc. for $C_{15}H_{20}N_2O_6$: C, 55.55; H, 6.17; N, 8.64 %

$[\alpha]_D^{25}$: -7.8 (c, 3.7, $CHCl_3$).

Z-Ser-Phe-OMe (53): (50%)

mp. : 102-104°C (lit.¹³⁵ mp. 79-80°C)

ir : $\nu_{max}(KBr)cm^{-1}$: 3300 (NH), 1732 (ester), 1688 (carbamate),
1650 (amide I), 1528 (amide II), 1450.

nmr : δ ($CDCl_3$): 3.02 (2H, brd, Phe $C^{\beta}H_2$), 3.61 (5H, s+m, $COOCH_3$
+ Ser $C^{\beta}H_2$), 4.22 (1H, m, Ser $C^{\alpha}H$), 4.79 (1H, m, Phe $C^{\alpha}H$),
5.03 (2H, s, Z CH_2), 6.05 (1H, d, J=7.5 Hz, Ser NH),
6.87-7.43 (11H, s + m, Phe NH + aromatic protons).

anal: Found: C, 62.74; H, 6.11; N, 7.26 %

Calc. for $C_{21}H_{24}N_2O_6$: C, 63.00; H, 6.00; N, 7.00 %

$[\alpha]_D^{25}$: -2.7 (c, 3.3, MeOH).

Z-Ser-Leu-OMe (55): (84%)

mp. : 77-78°C (lit.¹³⁷ mp. 73-74.5°C)

ir : $\nu_{max}(KBr)cm^{-1}$: 3400 (OH), 3310 (NH), 1745 (ester), 1695
(carbamate), 1660 (amide I), 1645 (amide I), 1550 (amide
II).

nmr : δ ($CDCl_3$): 0.84 (6H, d, J=5.0 Hz, Leu $CH_3 \times 2$), 1.53 (3H, m,
Leu $C^{\beta}H_2$ + Leu $C^{\gamma}H$), 3.71 (5H, s + m, $COOCH_3$ + Ser $C^{\beta}H_2$),
4.00-4.62 (2H, m, Leu $C^{\alpha}H$ + Ser $C^{\alpha}H$), 5.10 (2H, s, Z CH_2),
5.90 (1H, d, J=7.5 Hz, Ser NH), 7.03 (1H, d, J=7.5 Hz, Leu
NH), 7.37 (5H, s, aromatic protons).

anal: Found: C, 59.33; H, 7.18; N, 7.43 %

Calc. for $C_{18}H_{26}N_2O_6$: C, 59.02; H, 7.10; N, 7.65 %

$[\alpha]_D^{25}$: -32.65 (c, 1.66, MeOH).

Z-Ser-Tyr-OMe (59): (60%)

ir : ν_{\max} (KBr) cm^{-1} : 3397 (OH), 3315 (NH), 1750 (ester), 1708 (carbamate), 1649 (amide I), 1570, 1515 (amide II).

nmr : δ (CDCl_3): 3.00 (2H, m, Tyr C^βH_2), 3.71 (5H, s + m, COOCH_3 + Ser C^βH_2), 4.20 (1H, m, Ser C^αH), 4.60 (1H, m, Tyr C^αH), 5.06 (2H, s, Z CH_2), 5.84 (1H, d, $J=7.5$ Hz, Ser NH), 6.50 (1H, brd, Tyr NH), 6.66-7.12 (4H, dd, Tyr ring protons), 7.31 (5H, s, aromatic protons).

anal: Found: C, 60.36; H, 5.68; N, 6.48 %

Calc. for $\text{C}_{21}\text{H}_{24}\text{N}_2\text{O}_7$: C, 60.58; H, 5.77; N, 6.73 %

$[\alpha]_D^{25}$: +3.66 (c, 3.33, MeOH).

Z-Ser-Trp-OMe (61): (71%)

mp. : foamy solid (lit.¹⁴¹ mp. 101.5-103.5°C)

ir : ν_{\max} (KBr) cm^{-1} : 3350 (NH), 1720 (ester), 1655 (amide I), 1508 (amide II), 1450.

nmr : δ (CDCl_3): 3.26 (2H, d, $J=5.0$ Hz, Trp C^βH_2), 3.71 (5H, s + m, COOCH_3 + Ser C^βH_2), 4.25 (1H, m, Ser C^αH), 4.81-5.25 (3H, s + m, Trp C^αH + Z CH_2), 6.03 (1H, d, $J=7.5$ Hz, Ser NH), 6.89-7.57 (11H, s + m, Trp NH + aromatic protons), 8.64 (1H, brs, Indole NH).

ms : m/z : 439 (M)⁺.

anal: Found: C, 62.86; H, 5.34; N, 9.65 %

Calc. for $\text{C}_{23}\text{H}_{25}\text{N}_3\text{O}_6$: C, 62.87; H, 5.69; N, 9.57 %

$[\alpha]_D^{25}$: +9.38 (c, 0.81, MeOH).

Z-Ser-Asp(β -OMe)-OMe (64): (58%)

mp. : 97-98°C

ir : ν_{\max} (KBr) cm^{-1} : 3314 (NH), 1727 (ester), 1685 (carbamate), 1649 (amide I), 1549 (amide II), 1526 (amide II).

nmr : δ (60 MHz, CDCl_3): 2.90 (2H, d, $J=5.5$ Hz, Asp C^βH_2), 3.63, 3.73 (3H, 3H, s, s, $\text{COOCH}_3 \times 2$), 3.90 (2H, m, Ser C^βH_2), 4.16

(1H, m, Ser C^αH), 4.76 (1H, m, Asp C^αH), 5.10 (2H, s, Z CH₂), 5.83 (1H, br, Ser NH), 7.26 (6H, s + m, Asp NH + aromatic protons).

anal: Found: C, 53.78; H, 5.57; N, 7.23 %

Calc. for C₁₇H₂₂N₂O₈: C, 53.40; H, 5.76; N, 7.33 %

[α]_D²⁵: -14.77 (c, 3.33, MeOH).

Z-Ser-Ser-OMe (66): (60%)

mp. : 136-139°C (lit.¹⁴⁵ mp. 143-145°C)

ir : ν_{max} (KBr)cm⁻¹: 3445 (OH), 3305 (NH), 3280 (NH), 1738 (ester), 1663 (amide I), 1636 (amide I), 1547 (amide II).

nmr : δ [CDCl₃ + (CD₃)₂SO]: 3.68-3.93 (7H, s + br, Ser C^βH₂ × 2 + COOCH₃), 4.44 (2H, m, Ser C^αH × 2), 5.04 (2H, s, Z CH₂), 6.44 (1H, d, J=7.5 Hz, Ser NH(Z)), 7.25 (5H, s, aromatic protons), 7.61 (1H, d, J=7.5 Hz, Ser NH).

anal: Found: C, 53.08; H, 5.96; N, 8.41 %

Calc. for C₁₅H₂₀N₂O₇: C, 52.94; H, 5.88; N, 8.23 %

[α]_D²⁵: -4.2 (c, 3.3, MeOH).

Z-Ser-Met-OMe (67): (63%)

mp. : 98-99°C (lit.¹⁴⁷ mp. 101-102°C)

ir : ν_{max} (KBr)cm⁻¹: 3304 (NH), 1756 (ester), 1695 (carbamate), 1656 (amide I), 1545 (amide II).

nmr : δ (60 MHz, CDCl₃): 2.06 (5H, s + m, Met C^βH₂ + Met S-CH₃), 2.46 (2H, m, Met C^γH₂), 3.73 (3H, s, COOCH₃), 3.86 (2H, m, Ser C^βH₂), 4.20 (1H, m, Ser C^αH), 4.63 (1H, m, Met C^αH), 5.01 (2H, s, Z CH₂), 5.86 (1H, brd, Ser NH), 7.43 (6H, s + m, Met NH + aromatic protons).

anal: Found: C, 53.52; H, 6.27; N, 7.63 %

Calc. for C₁₇H₂₄N₂O₆S: C, 53.12; H, 6.25; N, 7.29 %

[α]_D²⁵: -25.42 (c, 1.66, MeOH).

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By a similar procedure anthranilic acid methyl ester and Pro-OMe were condensed with Z-Ser to give (57) and (62) (Chart C.4.b).

Z-Ser-Methylanthranilate (57): (43%)

mp. : 100-101°C

ir : ν_{\max} (KBr) cm^{-1} : 3459 (OH), 3391 (NH), 3293 (NH), 1708 (ester), 1680 (carbamate), 1626 (amide I), 1606, 1588, 1519 (amide II).

nmr : δ (CDCl_3): 3.90 (3H, s, COOCH_3), 4.16 (2H, m, Ser C^βH_2), 4.47 (1H, m, Ser C^αH), 5.20 (2H, s, Z CH_2), 5.84 (1H, brd, Ser NH), 7.03-7.66 (8H, m, aromatic protons + anthranilic NH + anthranilic ring protons x2), 8.06, 8.69 (1H, 1H, dd, dd, $J=7.5$ Hz, 1.25 Hz, anthranilic ring protons).

anal: Found: C, 60.82; H, 4.86; N, 7.78 %

Calc. for $\text{C}_{19}\text{H}_{20}\text{N}_2\text{O}_6$: C, 61.29; H, 5.38; N, 7.53 %

Z-Ser-Pro-OMe (62): (40%)

mp. : 113-115 (lit.¹⁴³ mp. 117-121°C)

ir : ν_{\max} (KBr) cm^{-1} : 3396 (OH), 3280 (NH), 1735 (ester), 1713 (carbamate), 1617 (amide I), 1558 (amide II), 1531 (amide II).

nmr : δ (CDCl_3): 2.09 (4H, m, Pro C^βH_2 + Pro $\text{C}^\gamma\text{H}_2$), 3.50-4.03 (7H, s + m, COOCH_3 + Pro $\text{C}^\delta\text{H}_2$ + Ser C^βH_2), 4.66 (2H, m, Pro C^αH + Ser C^αH), 5.19 (2H, s, Z CH_2), 5.78 (1H, brd, Ser NH), 7.42 (5H, s, aromatic protons).

anal: Found: C, 58.34; H, 6.16; N, 8.34 %

Calc. for $\text{C}_{17}\text{H}_{22}\text{N}_2\text{O}_6$: C, 58.28; H, 6.28; N, 8.00 %

$[\alpha]_D^{25}$: -79.15 (c, 1.66, MeOH).

Z-Ser-Alb-OMe — prepared by condensation of Z-Ser with *in situ* generated Alb-OMe (HOBt/DCC) — on treatment with ethanolic hydrazine hydrate at rt afforded the hydrazide which on condensation with Ser-OMe by

the azide coupling route afforded Z-Ser-Aib-Ser-OMe (69). By similar procedures Z-Ser-Leu-Ser-OMe (71), Z-Ser-Gly-Ser-OMe (73) and Z-Ser-Pro-Ser-OMe (75) were prepared (Chart C.4.c).

Z-Ser-Aib-Ser-OMe (69): (47%)

mp. : 166-168°C

ir : ν_{\max} (KBr) cm^{-1} : 3441 (OH), 3306 (NH), 3275 (NH), 1749 (ester), 1671 (carbamate), 1648 (amide I), 1627 (amide I), 1560 (amide II).

nmr : δ (CDCl_3): 1.53 (6H, s, s, Aib $\text{CH}_3 \times 2$), 3.84 (3H, s, COOCH_3), 4.03 (4H, m, Ser $\text{C}^\beta\text{H}_2 \times 2$), 4.20 (1H, m, Ser C^αH), 4.64 (1H, m, Ser C^αH), 5.22 (2H, s, Z CH_2), 6.03 (1H, d, $J=7.5$ Hz, Ser NH(Z)), 7.00 (1H, s, Aib NH), 7.15 (1H, m, Ser NH), 7.46 (5H, s, aromatic protons).

anal: Found: C, 53.27; H, 6.53; N, 9.64 %

Calc. for $\text{C}_{19}\text{H}_{27}\text{N}_3\text{O}_8$: C, 53.65; H, 6.35; N, 9.88 %

Z-Ser-Leu-Ser-OMe (71): (60%)

mp. : 182-183°C

ir : ν_{\max} (KBr) cm^{-1} : 3440 (OH), 3280 (NH), 1735 (ester), 1683 (carbamate), 1638 (amide I), 1615, 1535 (amide II).

nmr : δ [$\text{CDCl}_3 + (\text{CD}_3)_2\text{SO}$]: 0.87 (6H, d, $J=5.0$ Hz, Leu $\text{CH}_3 \times 2$), 1.62 (3H, m, Leu $\text{C}^\beta\text{H}_2 + \text{Leu } \text{C}^\gamma\text{H}$), 3.71 (7H, s + m, $\text{COOCH}_3 + \text{Ser } \text{C}^\beta\text{H}_2 \times 2$), 4.12-4.89 (3H, m, Leu $\text{C}^\alpha\text{H} + \text{Ser } \text{C}^\alpha\text{H} \times 2$), 5.09 (2H, s, Z CH_2), 6.71 (1H, d, $J=7.5$ Hz, Ser NH(Z)), 7.39 (5H, s, aromatic protons), 7.85 (2H, m, Leu NH + Ser NH).

ms : m/z : 454 (MH)⁺.

anal: Found: C, 55.37; H, 6.48; N, 9.38 %

Calc. for $\text{C}_{21}\text{H}_{31}\text{N}_3\text{O}_8$: C, 55.63; H, 6.84; N, 9.27 %

$[\alpha]_{\text{D}}^{25}$: -39.25 (c, 0.21, MeOH).

Z-Ser-Gly-Ser-OMe (73): (60%)

mp. : 171-172°C (crystallized from MeOH; lit.¹⁴⁸ mp. 173°C)

ir : ν_{\max} (KBr) cm^{-1} : 3470 (OH), 3390 (NH), 3315 (NH), 1732 (ester), 1680 (carbamate), 1650 (amide I), 1548 (amide II), 1512.

nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 3.56-4.03 (9H, s + m, COOCH_3 + Ser $\text{C}^\beta\text{H}_2 \times 2$ + Gly CH_2), 4.37-4.87 (2H, m, Ser $\text{C}^\alpha\text{H} \times 2$), 5.06 (2H, s, Z CH_2), 6.78 (1H, br, exchangeable with D_2O , Ser NH(Z)), 7.31 (5H, s, aromatic protons), 7.75 (1H, d, $J=7.5$ Hz, exchangeable, Ser NH), 8.09 (1H, t, exchangeable, Gly NH).

anal: Found: C, 51.37; H, 5.43; N, 10.36 %

Calc. for $\text{C}_{17}\text{H}_{23}\text{N}_3\text{O}_8$: C, 51.38; H, 5.79; N, 10.58 %

$[\alpha]_{\text{D}}^{25}$: -11.20 (c, 0.5, MeOH).

Z-Ser-Pro-Ser-OMe (75): (30%)

mp. : syrup

ir : ν_{\max} (KBr) cm^{-1} : 3381 (NH), 1742 (ester), 1719 (carbamate), 1639 (amide I), 1533 (amide II), 1452.

nmr : δ (CDCl_3): 2.00 (4H, br, Pro C^βH_2 + Pro $\text{C}^\gamma\text{H}_2$), 3.07-4.00 (9H, s+m, COOCH_3 + Pro $\text{C}^\delta\text{H}_2$ + Ser $\text{C}^\beta\text{H}_2 \times 2$), 4.53 (3H, m, Pro C^αH + Ser $\text{C}^\alpha\text{H} \times 2$), 5.03 (2H, s, Z CH_2), 6.29 (1H, d, $J=7.5$ Hz, exchangeable with D_2O , Ser NH(Z)), 7.28 (5H, s, aromatic protons), 7.68 (1H, d, $J=7.5$ Hz, exchangeable, Ser NH).

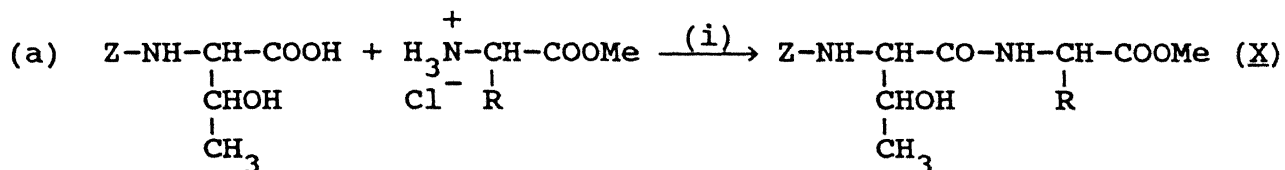
anal: Found: C, 55.19; H, 6.33; N, 9.45 %

Calc. for $\text{C}_{20}\text{H}_{27}\text{N}_3\text{O}_8$: C, 54.92; H, 6.18; N, 9.61 %

$[\alpha]_{\text{D}}^{25}$: -65.53 (c, 3.16, MeOH).

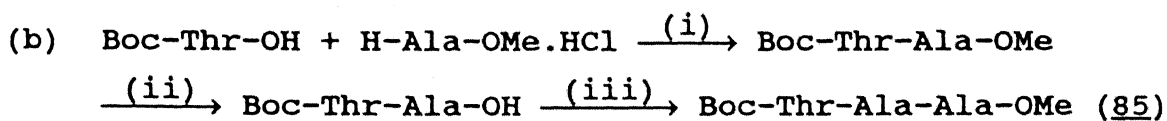
Dipeptides having N-terminal Thr residues were prepared by condensation with a range of, *in situ* generated, α -amino acid esters using HOBT/DCC in CH_2Cl_2 -DMF (Chart C.5.a).

CHART C.5



<u>R</u>	<u>(X)</u>
H	Z-Thr-Gly-OMe (<u>78</u>)
CH ₃	Z-Thr-Ala-OMe (<u>79</u>)
CH ₂ Ph	Z-Thr-Phe-OMe (<u>80</u>)
CH ₂ CH(CH ₃) ₂	Z-Thr-Leu-OMe (<u>81</u>)
CH(OH)CH ₃	Z-Thr-Thr-OMe (<u>82</u>)
(CH ₂) ₄ NHZ	Z-Thr-Lys(N ^ω Z)-OMe (<u>83</u>)
CH ₂ SCH ₂ Ph	Z-Thr-Cys(S-Bzl)-OMe (<u>89</u>)

(i) HOBT/DCC/CH₂Cl₂-DMF/NEt₃

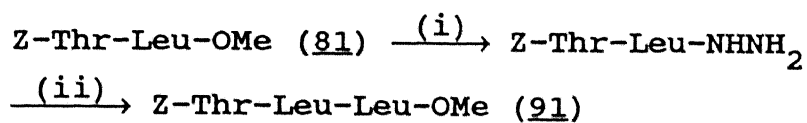
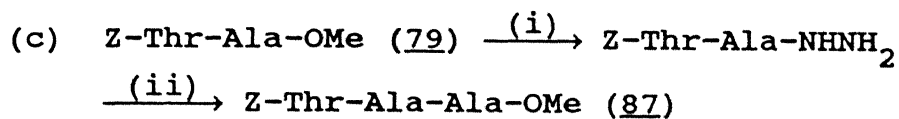


(i) HOBT/DCC/CH₂Cl₂-DMF/NEt₃;

(ii) 2N NaOH-MeOH/0°/rt/4 h;

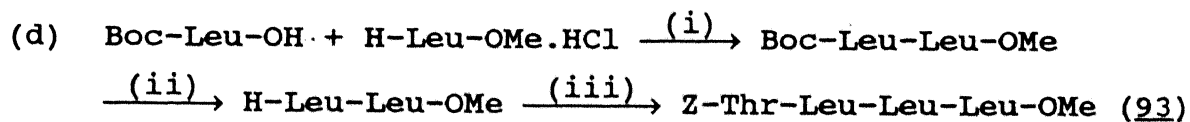
(iii) HOBT/DCC/CH₂Cl₂-DMF; H-Ala-OMe.HCl/NEt₃

CHART C.5 (CONTINUED)



(i) NH₂NH₂·H₂O/EtOH/rt/24 h;

(ii) Aq. AcOH/6N HCl/NaNO₂/0°; H-Ala-OMe.HCl or H-Leu-OMe.HCl/
 NEt₃/CH₂Cl₂



(i) HOBt/DCC/CH₂Cl₂-DMF/NEt₃;

(ii) TFA/CH₂Cl₂/0°/2 h;

(iii) Z-Thr-Leu-NHNH₂/Aq. AcOH/6N HCl/NaNO₂/0°; NEt₃/CH₂Cl₂/0°

Z-Thr-Gly-OMe (78): (78%)

mp. : 94-97°C (lit.¹⁵⁰ mp. 105-106°C)

ir : ν_{\max} (KBr) cm^{-1} : 3288 (NH), 1731 (ester), 1689 (carbamate),
1649 (amide I), 1556 (amide II).

nmr : δ (CDCl_3): 1.15 (3H, d, $J=6.5$ Hz, Thr CH_3), 3.71 (3H, s, COOCH_3), 3.92 (2H, d, $J=5.0$ Hz, Gly CH_2), 4.21 (2H, m, Thr C^αH + Thr C^βH), 5.09 (2H, s, Z CH_2), 6.06 (1H, d, $J=7.5$ Hz, Thr NH), 7.34 (6H, s + m, Gly NH + aromatic protons).

anal: Found: C, 55.09; H, 6.42; N, 8.36 %

Calc. for $\text{C}_{15}\text{H}_{20}\text{N}_2\text{O}_6$: C, 55.55; H, 6.17; N, 8.64 %

 $[\alpha]_{\text{D}}^{25}$: -12.3 (c, 2.2, MeOH).

Z-Thr-Ala-OMe (79): (60%)

mp. : 127-128°C (lit.¹⁵¹ mp. 132-134°C)

ir : ν_{\max} (KBr) cm^{-1} : 3404 (OH), 3300 (NH), 3065, 1756 (ester),
1692 (carbamate), 1648 (amide I), 1542 (amide II).

nmr : δ (CDCl_3): 1.14 (3H, d, $J=6.5$ Hz, Thr CH_3), 1.34 (3H, d, $J=7.0$ Hz, Ala CH_3), 3.75 (3H, s, COOCH_3), 4.22 (2H, m, Thr C^αH + Thr C^βH), 4.53 (1H, m, Ala C^αH), 5.12 (2H, s, Z CH_2), 6.00 (1H, d, $J=7.5$ Hz, Thr NH), 7.35 (6H, s + m, Ala NH + aromatic protons).

anal: Found: C, 57.09; H, 6.43; N, 8.38 %

Calc. for $\text{C}_{16}\text{H}_{22}\text{N}_2\text{O}_6$: C, 56.80; H, 6.51; N, 8.28 %

 $[\alpha]_{\text{D}}^{25}$: -28.3 (c, 3.3, MeOH).

Z-Thr-Phe-OMe (80): (63%)

mp. : 98-99°C (lit.¹⁵² mp. 105-106°C)

ir : ν_{\max} (KBr) cm^{-1} : 3299 (NH), 3063, 1743 (ester), 1697
(carbamate), 1648 (amide I), 1540 (amide II).

nmr : δ (CDCl_3): 1.09 (3H, d, $J=6.5$ Hz, Thr CH_3), 3.06 (2H, m, Phe C^βH_2), 3.68 (3H, s, COOCH_3), 4.15 (2H, m, Thr C^αH + Thr

Z-Thr-Lys(N^ωZ)-OMe (83): (59%)

mp. : 98-99°C (lit.¹⁵⁵)

ir : ν_{\max} (KBr)cm⁻¹: 3320 (NH), 1742 (ester), 1686 (carbamate),
1649 (amide I), 1541 (amide II).

nmr : δ (CDCl₃): 1.12 (3H, d, J=6.5 Hz, Thr CH₃), 1.40 (4H, m, Lys C^βH₂ + Lys C^γH₂), 1.68 (2H, m, Lys C^δH₂), 3.12 (2H, m, Lys C^ωH₂), 3.71 (3H, s, COOCH₃), 4.04-4.60 (3H, m, Thr C^αH + Thr C^βH + Lys C^αH), 5.04, 5.11 (2H, 2H, s, s, Z CH₂×2), 5.78 (1H, d, J=7.5 Hz, Thr NH), 7.00 (1H, d, J=7.5 Hz, Lys N^ωH), 7.34 (11H, s + m, Lys NH + aromatic protons).

ms : m/z: 530 (MH)⁺.

anal: Found: C, 61.44; H, 6.23; N, 7.88 %

Calc. for C₂₇H₃₅N₃O₈: C, 61.25; H, 6.62; N, 7.94 %

[α]_D²⁵: -12.29 (c, 1.66, MeOH)

Z-Thr-Cys(S-Bzl)-OMe (89): (90%)

mp. : 140-141°C

ir : ν_{\max} (KBr)cm⁻¹: 3306 (NH), 1744 (ester), 1694 (carbamate),
1644 (amide I), 1530 (amide II).

nmr : δ (CDCl₃): 1.12 (3H, d, J=6.5 Hz, Thr CH₃), 2.81 (2H, m, Cys C^βH₂), 3.69, 3.72 (5H, s, s, COOCH₃ + Bzl CH₂S), 3.93-4.50 (2H, m, Thr C^αH + Thr C^βH), 4.71 (1H, m, Cys C^αH), 5.12 (2H, s, Z CH₂), 5.68 (1H, d, J=7.5 Hz, Thr NH), 7.12-7.56 (11H, s, Cys NH, aromatic protons).

ms : m/z: 461 (MH)⁺.

anal: Found: C, 59.87; H, 5.83; N, 6.38 %

Calc. for C₂₃H₂₈N₂O₆S: C, 60.00; H, 6.09; N, 6.09 %

[α]_D²⁵: -26.14 (c, 0.83, MeOH).

Boc-Thr-Ala-Ala-OMe (85) was prepared by sequential condensation from Boc-Thr as illustrated in Chart C.5.b.

Boc-Thr-Ala-Ala-OMe (85): (45%)

mp. : 127-128°C

ir : ν_{\max} (KBr) cm^{-1} : 3325 (NH), 1737 (ester), 1702 (carbamate), 1676 (amide I), 1635 (amide I), 1543 (amide II).

nmr : δ (CDCl_3): 1.15 (3H, d, $J=6.5$ Hz, Thr CH_3), 1.37 (6H, d, $J=7.5$ Hz, Ala $\text{CH}_3 \times 2$), 1.43 (9H, s, Boc $\text{CH}_3 \times 3$), 3.73 (3H, s, COOCH_3), 4.00-4.72 (4H, m, Thr C^αH + Thr C^βH + Ala $\text{C}^\alpha\text{H} \times 2$), 5.53 (1H, d, $J=7.5$ Hz, Thr NH), 7.00 (2H, m, Ala $\text{NH} \times 2$).

anal: Found: C, 50.89; H, 7.67; N, 11.28 %

Calc. for $\text{C}_{16}\text{H}_{29}\text{N}_3\text{O}_7$: C, 51.20; H, 7.73; N, 11.20 %

Z-Thr-Ala-Ala-OMe (87) was prepared from Z-Thr-Ala-NHNH₂ via azide coupling with *in situ* generated Leu-OMe. The Z-Thr-Ala-NHNH₂, in turn, was readily obtained from Z-Thr-Ala-OMe (79). By a similar procedure Z-Thr-Leu-OMe (81) was transformed to Z-Thr-Leu-Leu-OMe (91) (Chart C.5.c).

Z-Thr-Ala-Ala-OMe (87): (42%)

mp. : 165-166°C

ir : ν_{\max} (KBr) cm^{-1} : 3296 (NH), 1738 (ester), 1697 (carbamate), 1636 (amide I), 1551 (amide II),

nmr : δ (CDCl_3): 1.15 (3H, d, $J=6.5$ Hz, Thr CH_3), 1.34 (6H, d, $J=7.5$ Hz, Ala $\text{CH}_3 \times 2$), 3.75 (3H, s, COOCH_3), 4.09-4.68 (4H, m, Thr C^αH + Thr C^βH + Ala $\text{C}^\alpha\text{H} \times 2$), 5.12 (2H, s, Z CH_2), 5.78 (1H, d, $J=7.5$ Hz, Thr NH), 7.34 (5H, s, aromatic protons), 7.84 (2H, m, Ala $\text{NH} \times 2$).

anal: Found: C, 55.92; H, 6.72; N, 10.38 %

Calc. for $\text{C}_{19}\text{H}_{27}\text{N}_3\text{O}_7$: C, 55.74; H, 6.60; N, 10.27 %

$[\alpha]_D^{25}$: -51.60 (c, 0.56, MeOH).

Z-Thr-Leu-Leu-OMe (91): (59%)

mp. : 136-137°C

ir : ν_{\max} (KBr) cm^{-1} : 3290 (NH), 1746 (ester), 1699 (carbamate), 1640 (amide I), 1542 (amide II).

nmr : δ (60 MHz, CDCl_3): 0.90 (12H, brs, Leu $\text{CH}_3 \times 4$), 1.13 (3H, d, $J=6.5$ Hz, Thr CH_3), 1.59 (6H, m, Leu $\text{C}^\beta\text{H}_2 \times 2$ + Leu $\text{C}^\gamma\text{H} \times 2$), 3.66 (3H, s, COOCH_3), 4.03-4.76 (4H, m, Thr C^αH + Thr C^βH + Leu $\text{C}^\alpha\text{H} \times 2$), 5.06 (2H, s, Z CH_2), 5.96 (1H, brd, Thr NH), 6.73-7.46 (7H, s+m, Leu $\text{NH} \times 2$ + aromatic protons).

ms : m/z : 494 (MH)⁺.

anal: Found: C, 61.38; H, 7.84; N, 8.09 %

Calc. for $\text{C}_{25}\text{H}_{39}\text{N}_3\text{O}_7$: C, 60.85; H, 7.91; N, 8.52 %

$[\alpha]_D^{25}$: -45.84 (c, 1.66, MeOH).

The N-terminal Thr containing tetrapeptide Z-Thr-Leu-Leu-Leu-OMe (93) was obtained by azide condensation procedure involving Z-Thr-Leu-NHNH₂ and H-Leu-Leu-OMe (Chart C.5.d).

Z-Thr-Leu-Leu-Leu-OMe (93): (52%)

mp. : 197-198°C

ir : ν_{max} (KBr) cm^{-1} : 3281 (NH), 1750 (ester), 1699 (carbamate), 1637 (amide I), 1543 (amide II).

nmr : δ (CDCl_3): 0.89 (18H, brs, Leu $\text{CH}_3 \times 6$), 1.11 (3H, d, $J=6.5$ Hz, Thr CH_3), 1.66 (9H, m, Leu $\text{C}^\beta\text{H}_2 \times 3$ + Leu $\text{C}^\gamma\text{H} \times 3$), 3.69 (3H, s, COOCH_3), 3.93-4.84 (5H, m, Thr C^αH + Thr C^βH + Leu $\text{C}^\alpha\text{H} \times 3$), 5.09 (2H, s, Z CH_2), 6.03 (1H, d, $J=7.5$ Hz, Thr NH), 7.43 (8H, s+m, Leu $\text{NH} \times 3$ + aromatic protons).

ms : m/z : 607 (MH)⁺.

anal: Found: C, 61.44; H, 8.08; N, 9.43 %

Calc. for $\text{C}_{31}\text{H}_{50}\text{N}_4\text{O}_8$: C, 61.39; H, 8.25; N, 9.24 %

$[\alpha]_D^{25}$: -61.5 (c, 1.66, MeOH).

The C-terminal ester function of serine and threonine containing amino acid and peptide substrates were smoothly transformed to the corresponding C-terminal amides in methanolic ammonia. These compounds were used to assess the role of the C-terminal ending on the course of

Ru(VIII) mediated scission of Ser/Thr residue containing peptides (Chart C.6).

Bz-Ser-NH₂ (95): (75%)

mp. : 162-163°C

ir : ν_{\max} (KBr)cm⁻¹: 3378 (NH), 3292 (NH), 3188 (NH), 1634 (amide I), 1578, 1525 (amide II).

Z-Ser-NH₂ (97): (82%)

mp. : 130-131°C (lit.¹⁵⁷ mp. 132°C)

ir : ν_{\max} (KBr)cm⁻¹: 3376 (OH), 3318 (NH), 3204 (NH), 1686, 1650 (amide I), 1532 (amide II), 1465.

nmr : δ [(CD₃)₂SO]: 3.65 (2H, m, Ser C ^{β} H₂), 4.06 (1H, m, Ser C ^{α} H), 5.06 (2H, s, Z CH₂) 6.56-7.46 (8H, s + m, Ser NH + CONH₂ + aromatic protons).

anal: Found: C, 55.65; H, 5.96; N, 11.89 %

Calc. for C₁₁H₁₄N₂O₄: C, 55.46; H, 5.88; N, 11.76 %

[α]_D²⁶: +7.95 (c, 1.66, MeOH).

Z-Leu-Ser-NH₂ (98): (74%)

mp. : 148-149°C

ir : ν_{\max} (KBr)cm⁻¹: 3299 (NH), 1689, 1646 (amide I), 1540 (amide II).

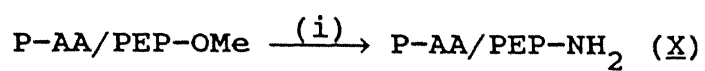
nmr : δ [CDCl₃ + (CD₃)₂SO]: 0.87 (6H, d, J=5.0 Hz, Leu CH₃x2), 1.56 (3H, m, Leu C ^{β} H₂ + Leu C ^{γ} H), 3.68 (2H, m, Ser C ^{β} H₂), 4.15 (1H, m, Ser C ^{α} H), 4.80 (1H, m, Leu C ^{α} H), 5.06 (2H, s, Z CH₂), 6.78-7.37 (8H, s + m, Leu NH + CONH₂ + aromatic protons), 7.65 (1H, d, J=7.5 Hz, Ser NH).

anal: Found: C, 58.24; H, 7.34; N, 11.8 %

Calc. for C₁₇H₂₅N₃O₅: C, 58.12; H, 7.12; N, 11.97 %

Bz-Gly-Ser-NH₂ (100): (65%)

mp. : 110-111°C

CHART C.6

AA = Amino Acid, PEP = Peptide

(X)

Bz-Ser-NH₂ (95)

Z-Ser-NH₂ (97)

Z-Leu-Ser-NH₂ (98)

Bz-Gly-Ser-NH₂ (100)

Z-Thr-NH₂ (102)

Z-Ser-Leu-NH₂ (103)

Z-Ser-Ser-NH₂ (104)

(i) NH₃/MeOH/0°/12 h

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3382 (NH), 3283 (NH), 1661 (amide I), 1552 (amide II).

nmr : δ [$\text{CDCl}_3 + (\text{CD}_3)_2\text{SO}$]: 3.78 (2H, m, Ser C^βH_2), 3.96 (2H, d, $J=5.0$ Hz, Gly CH_2), 4.39 (1H, m, Ser C^αH), 4.80 (1H, br, exchangeable with D_2O , Ser OH), 6.84 (1H, br, Gly NH), 7.09-8.60 (8H, m, Ser NH + CONH_2 + aromatic protons).

anal: Found: C, 54.52; H, 5.72; N, 15.89 %

Calc. for $\text{C}_{12}\text{H}_{15}\text{N}_3\text{O}_4$: C, 54.34; H, 5.66; N, 15.85 %

Z-Thr- NH_2 (102): (60%)

mp. : sticky solid

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3280 (br, NH), 1670 (amide I), 1638 (amide I), 1600, 1525 (br, amide II).

Z-Ser-Leu- NH_2 (103): (90%)

mp. : 110-115°

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3444 (OH), 3303 (NH), 1644 (br, amide I), 1541 (amide II).

anal: Found: C, 58.30; H, 7.29; N, 11.72 %

Calc. for $\text{C}_{17}\text{H}_{25}\text{N}_3\text{O}_5$: C, 58.12; H, 7.12; N, 11.97 %

Z-Ser-Ser- NH_2 (104): (76%)

mp. : 221-222°C

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3401 (OH), 3297 (NH), 3268 (NH), 1685, 1647 (amide I), 1548 (amide II).

nmr : δ [$\text{CDCl}_3 + (\text{CD}_3)_2\text{SO}$]: 3.68 (4H, m, Ser $\text{C}^\beta\text{H}_2 \times 2$), 4.20 (2H, m, Ser $\text{C}^\alpha\text{H} \times 2$), 5.04 (2H, s, Z CH_2), 6.82 (2H, br, exchangeable, CONH_2), 7.10 (1H, br, exchangeable, Ser NH (Z)), 7.32 (5H, s, aromatic protons), 7.79 (1H, br, exchangeable, Ser NH).

MS : m/z : 326 (MH)⁺.

It may be noted that the compounds described thus far have Ser/Thr residues placed either at the C-terminal or the N-terminal locations. In order to complete the mural, these were augmented with peptides wherein the Ser/Thr residues were placed in non-terminal locations.

Tripeptides where the serine residue was placed in the middle location were prepared from C-terminal Ser-OMe dipeptide precursors via sequence, transformation to the hydrazide and azide coupling with the appropriate partner in the form of *in situ* generated amino acid ester. The tripeptides Bz-Leu-Ser-Leu-OMe (105), Bz-Ala-Ser-Ala-OMe (107), Z-Leu-Ser-His-OMe (109), Z-Gly-Ser-Gly-OMe (112), Bz-Pro-Ser-Pro-OMe (114) and Bz-Aib-Ser-Aib-OMe (116) were prepared using this approach (Chart C.7.a; Chart C.7.b).

Bz-Leu-Ser-Leu-OMe (105): (74%)

mp. : 87-88°C

ir : ν_{\max} (KBr) cm^{-1} : 3286 (NH), 1747 (ester), 1636 (amide I), 1532 (amide II).

nmr : δ (CDCl_3): 0.93 (12H, m, Leu $\text{CH}_3 \times 4$), 1.62 (6H, m, Leu $\text{C}^\beta \text{H}_2 \times 2$ + Leu $\text{C}^\gamma \text{H} \times 2$), 3.72 (3H, s, COOCH_3), 3.87 (2H, br, Ser $\text{C}^\beta \text{H}_2$), 4.37-5.00 (3H, m, Leu $\text{C}^\alpha \text{H} \times 2$ + Ser $\text{C}^\alpha \text{H}$), 7.06-7.96 (8H, m, Leu $\text{NH} \times 2$ + Ser NH + aromatic protons).

anal: Found: C, 61.16; H, 7.83; N, 9.48 %

Calc. for $\text{C}_{23}\text{H}_{35}\text{N}_3\text{O}_6$: C, 61.47; H, 7.79; N, 9.35 %

$[\alpha]_D^{26}$: -25.9 (c, 3.3, CHCl_3).

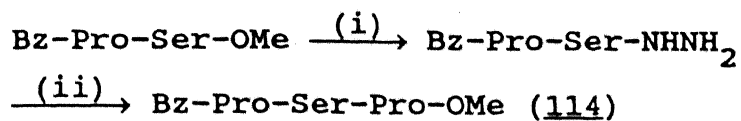
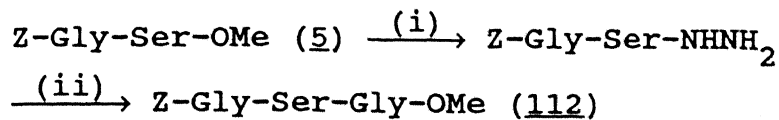
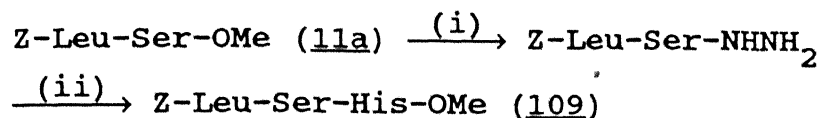
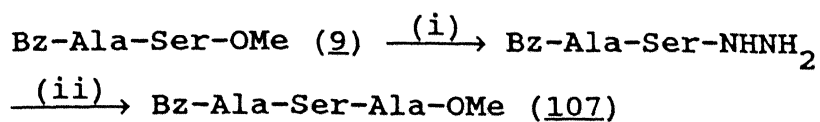
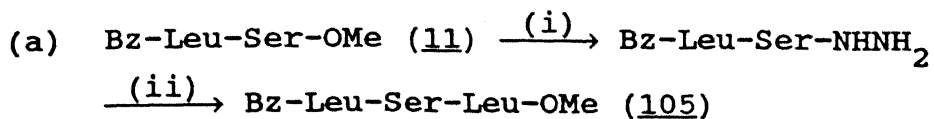
Bz-Ala-Ser-Ala-OMe (107): (62%)

mp. : 195-196°C

ir : ν_{\max} (KBr) cm^{-1} : 3270 (NH), 3070, 2980, 2930, 1743 (ester), 1688, 1625 (amide I), 1533 (amide II).

nmr : δ [400 MHz, CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 1.28, 1.36 (3H, 3H, d, d, $J=7.2$ Hz, 7.2 Hz, Ala $\text{CH}_3 \times 2$), 3.40 (2H, m, Ser $\text{C}^\beta \text{H}_2$), 3.60 (3H, s, COOCH_3), 4.28 (2H, m, Ala $\text{C}^\alpha \text{H} \times 2$), 4.48 (1H, m, Ser

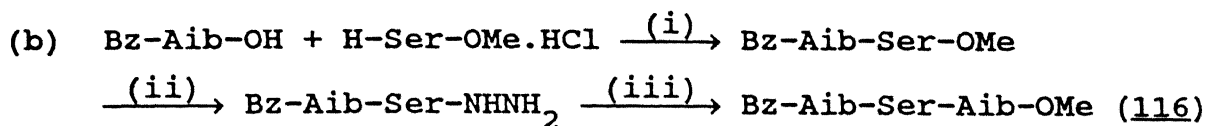
CHART C.7



(i) NH₂NH₂·H₂O/EtOH/rt/24 h;

(ii) Aq. AcOH/6N HCl/NaNO₂/0°; H-Leu-OMe.HCl or H-Ala-OMe.HCl
 or H-His-OMe.2HCl or H-Gly-OMe.HCl or H-Pro-OMe.HCl/NEt₃/
 CH₂Cl₂/0°

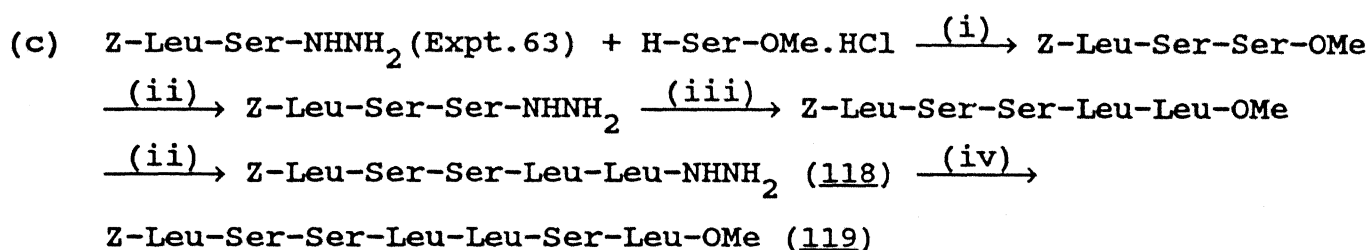
CHART C.7 (CONTINUED)



(i) $\text{HOBT/DCC/CH}_2\text{Cl}_2\text{-DMF/NEt}_3$;

(ii) $\text{NH}_2\text{NH}_2\cdot\text{H}_2\text{O/EtOH/rt/24 h}$;

(iii) $\text{Aq. AcOH/6N HCl/NaNO}_2/0^\circ$; $\text{H-Aib-OMe.HCl/NEt}_3/\text{CH}_2\text{Cl}_2$



(i) $\text{Aq. AcOH/6N HCl/NaNO}_2/0^\circ$; $\text{H-Ser-OMe.HCl/NEt}_3/\text{CH}_2\text{Cl}_2$;

(ii) $\text{NH}_2\text{NH}_2\cdot\text{H}_2\text{O/EtOH/rt/24 h}$;

(iii) $\text{Aq. AcOH/6N HCl/NaNO}_2/0^\circ$; $\text{H-Leu-Leu-OMe.HCl (Expt. 53)/NEt}_3/\text{CH}_2\text{Cl}_2$;

(iv) a. $\text{Z-Ser-Leu-OMe} \longrightarrow \text{H-Ser-Leu-OMe (Pd/C/5\%/EtOAc/4-6 h)}$;

b. $\text{Aq AcOH/6N HCl/NaNO}_2/0^\circ$; $\text{H-Ser-Leu-OMe/CH}_2\text{Cl}_2$

$C^{\alpha}H$), 7.50 (3H, m, aromatic protons), 7.90 (3H, m, NH + aromatic protons), 8.16 (1H, d, $J=7.5$ Hz, NH), 8.54 (1H, d, $J=7.5$ Hz, NH).

ms : m/z : 365 (M)⁺.

anal: Found: C, 55.54; H, 6.27; N, 11.59 %

Calc. for $C_{17}H_{23}N_3O_6$: C, 55.89; H, 6.30; N, 11.51 %

$[\alpha]_D^{26}$: -30.96 (c, 1.76, MeOH).

Z-Leu-Ser-His-OMe (109): (68%)

mp. : 126-127°C

ir : ν_{max} (KBr) cm^{-1} : 3298 (NH), 1732 (ester), 1688 (carbamate), 1640 (amide I), 1537 (amide II).

nmr : δ [$CDCl_3$ + $(CD_3)_2SO$]: 0.90 (6H, d, $J=5.0$ Hz, Leu $CH_3 \times 2$), 1.62 (3H, m, Leu $C^{\beta}H_2$ + Leu $C^{\gamma}H$), 3.06 (2H, m, His $C^{\beta}H_2$), 3.53-4.00 (5H, s + m, $COOCH_3$ + Ser $C^{\beta}H_2$), 4.37 (1H, m, Ser $C^{\alpha}H$), 4.53-5.37 (4H, s + m, Z CH_2 + Leu $C^{\alpha}H$ + His $C^{\alpha}H$), 6.50 (1H, d, $J=7.5$ Hz, Leu NH), 6.81 (1H, s, Imidazolyl 4H), 7.18-8.15 (8H, s + m, His NH + Ser NH + Imidazolyl 2H + aromatic protons).

ms : m/z : 504 (MH)⁺.

anal: Found: C, 57.43; H, 6.67; N, 13.49 %

Calc. for $C_{24}H_{33}N_5O_7$: C, 57.26; H, 6.56; N, 13.92 %

$[\alpha]_D^{26}$: -15.66 (c, 1.66, MeOH).

Z-Gly-Ser-Gly-OMe (112): (50%)

mp. : 154-155°C

ir : ν_{max} (KBr) cm^{-1} : 3297 (NH), 3066, 1760 (ester), 1704 (carbamate), 1646 (amide I), 1555 (amide II).

nmr : δ [$CDCl_3$ + $(CD_3)_2SO$]: 3.50-4.03 (9H, s + m, $COOCH_3$ + Ser $C^{\beta}H_2$ + Gly $CH_2 \times 2$), 4.53 (1H, m, Ser $C^{\alpha}H$), 5.12 (2H, s, Z CH_2), 7.00 (1H, br, Gly NH(Z)), 7.37 (5H, s, aromatic protons), 7.69 (1H, m, Gly NH), 8.00 (1H, m, Ser NH).

anal: Found: C, 52.49; H, 5.81; N, 11.38 %

Calc. for $C_{16}H_{21}N_3O_7$: C, 52.32; H, 5.72; N, 11.44 %

$[\alpha]_D^{26}$: -16.6 (c, 1.0, MeOH).

Bz-Pro-Ser-Pro-OMe (114): (44%)

mp. : 70-71°C

ir : ν_{\max} (KBr) cm^{-1} : 3340 (NH), 1735 (ester), 1625 (amide I),
1570 (amide II), 1530 (amide II), 1440.

nmr : δ (CDCl_3): 2.06 (8H, m, Pro $\text{C}^\beta\text{H}_2 \times 2$ + Pro $\text{C}^\gamma\text{H}_2 \times 2$),
3.40-4.03 (9H, s + m, COOCH_3 + Pro $\text{C}^\delta\text{H}_2 \times 2$ + Ser C^βH_2),
4.25-5.21 (3H, m, Ser C^αH + Pro $\text{C}^\alpha\text{H} \times 2$), 7.18-8.06 (6H, m,
Ser NH + aromatic protons).

anal: Found: C, 60.28; H, 6.35; N, 9.87 %

Calc. for $C_{21}H_{27}N_3O_6$: C, 60.43; H, 6.47; N, 10.07 %

$[\alpha]_D^{26}$: -42.52 (c, 1.74, CHCl_3).

Bz-Aib-Ser-Aib-OMe (116): (75%)

mp. : 73-74°C

ir : ν_{\max} (KBr) cm^{-1} : 3290 (NH), 1737 (ester), 1649 (amide I),
1537 (amide II).

nmr : δ (CDCl_3): 1.66 (12H, m, Aib $\text{CH}_3 \times 4$), 3.69 (3H, s, COOCH_3),
3.87-4.56 (3H, m, Ser C^βH_2 + Ser C^αH), 7.10-8.00 (8H, m,
Ser NH + Aib $\text{NH} \times 2$ + aromatic protons).

ms : m/z: 394 (MH)⁺.

anal: Found: C, 57.91; H, 6.56; N, 10.59 %

Calc. for $C_{19}H_{27}N_3O_6$: C, 58.01; H, 6.87; N, 10.69 %

$[\alpha]_D^{26}$: -6.89 (c, 2.13, EtOH).

From the vantage of $\text{C}^\alpha\text{-C}$ side chain scission of Ser residues, compound Z-Leu-Ser-Ser-Leu-Leu-Ser-Leu-OMe (119) provides an excellent substrate not only because of the presence of three serine residues here but also since two of these are placed contiguously. Compound (119) was prepared from the precursor hydrazide Z-Leu-Ser-Ser-Leu-Leu-NHNH₂ (118)

via procedure outlined in Chart C.7.c.

Z-Leu-Ser-Ser-Leu-Leu-NHNH₂ (118): (63%)

mp. : 172-173°C

ir : ν_{\max} (KBr)cm⁻¹: 3293 (br, NH), 1653 (br, amide I), 1541 (amide II).

nmr : δ [400 MHz, (CD₃)₂SO]: 0.82 (18H, m, Leu CH₃x6), 1.34-1.68 (9H, m, Leu C ^{β} H₂x3 + Leu C ^{γ} Hx3), 3.44-3.70 (4H, m, Ser C ^{β} H₂x2), 4.02-4.38 (5H, m, C ^{α} Hx5), 5.02 (2H, s, Z CH₂), 5.16 (1H, br, Leu NH(Z)), 7.02 (2H, m, NHx2), 7.34 (5H, s, aromatic protons), 7.48 (1H, d, J=7.5 Hz, NH), 7.60 (1H, d, J=7.5 Hz, NH), 8.00 (3H, m, NHx3).

Z-Leu-Ser-Ser-Leu-Leu-Ser-Leu-OMe (119): (41%)

mp. : 145-146°C

ir : ν_{\max} (KBr)cm⁻¹: 3291 (br, NH), 1748 (ester), 1694 (amide I), 1648 (amide I), 1534 (amide II).

nmr : δ [400 MHz, (CD₃)₂SO]: 0.93 (24H, brd, Leu CH₃x8), 1.66 (12H, m, Leu C ^{β} H₂x4 + Leu C ^{γ} Hx4), 3.73 (3H, brs, COOCH₃), 4.00 (2H, m, Ser C ^{β} H₂), 4.20 (4H, m, Ser C ^{β} H₂x2), 4.46 (4H, m, C ^{α} Hx4), 5.15 (5H, s+m, Z CH₂ + C ^{α} Hx3), 5.66 (1H, br, NH), 6.26 (1H, br, NH), 7.08-7.84 (9H, s+m, NHx4 + aromatic protons), 8.35 (1H, br, NH).

anal: Found: C, 57.44; H, 7.58; N, 11.36 %

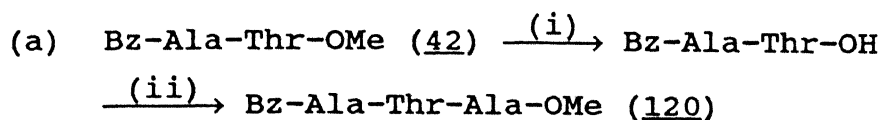
Calc. for C₄₂H₆₉N₇O₁₃: C, 57.34; H, 7.85; N, 11.15 %

Bz-Ala-Thr-Ala-OMe (120) and Bz-Pro-Thr-Pro-OMe (121) were prepared from the free C-terminal Thr precursors by HOBt/DCC mediated coupling with appropriate partners, generated *in situ* from hydrochloride precursors (Chart C.8.a and C.8.b).

Bz-Ala-Thr-Ala-OMe (120): (65%)

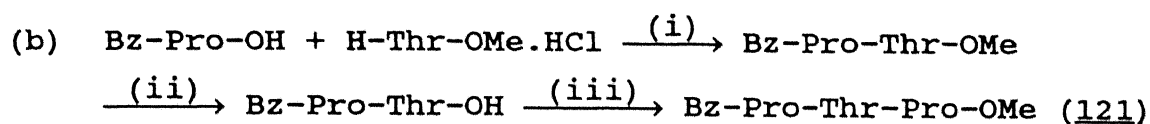
mp. : 206-207°C

CHART C.8



(i) 2N NaOH-MeOH/0°/rt/4 h;

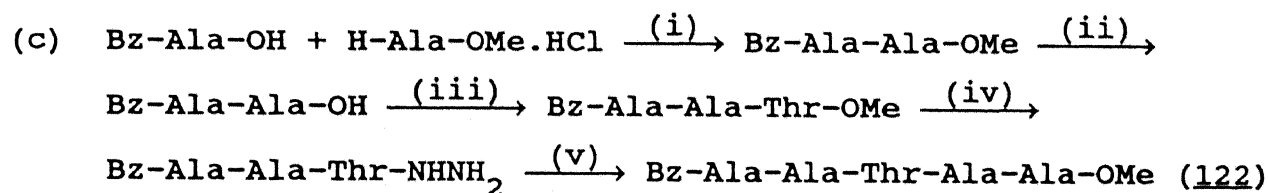
(ii) HOBT/DCC/CH₂Cl₂-DMF; H-Ala-OMe.HCl/NEt₃/CH₂Cl₂



(i) HOBT/DCC/CH₂Cl₂-DMF/NEt₃;

(ii) 2N NaOH-MeOH/0°/rt/4 h;

(iii) HOBT/DCC/CH₂Cl₂-DMF; H-Pro-OMe.HCl/NEt₃/CH₂Cl₂



(i) HOBT/DCC/CH₂Cl₂-DMF/NEt₃;

(ii) 2N NaOH-MeOH/0°/rt/4 h;

(iii) HOBT/DCC/CH₂Cl₂-DMF; H-Thr-OMe.HCl/NEt₃;

(iv) NH₂NH₂.H₂O/EtOH/rt/24 h;

(v) Aq. AcOH/6N HCl/NaNO₂/0°; H-Ala-Ala-OMe/CH₂Cl₂

ir : ν_{\max} (KBr) cm^{-1} : 3326 (NH), 3268 (NH), 1738 (ester), 1683 (amide I), 1623 (amide I), 1577 (amide II), 1532 (amide II).

nmr : δ (CDCl_3): 1.12 (3H, d, $J=6.5$ Hz, Thr CH_3), 1.40 (3H, d, $J=7.5$ Hz, Ala CH_3), 1.53 (3H, d, $J=7.5$ Hz, Ala CH_3), 3.78 (3H, s, COOCH_3), 4.15-4.96 (4H, m, Thr C^αH + Thr C^βH + Ala $\text{C}^\alpha\text{H}_2$), 7.31-8.22 (8H, m, Thr NH + Ala NH_2 + aromatic protons).

anal: Found: C, 57.14; H, 6.26; N, 10.87 %

Calc. for $\text{C}_{18}\text{H}_{25}\text{N}_3\text{O}_6$: C, 56.99; H, 6.60; N, 11.08 %

$[\alpha]_D^{26}$: -30.9 (c, 3.3, MeOH).

Bz-Pro-Thr-Pro-OMe (121): (83%)

mp. : syrup

ir : ν_{\max} (KBr) cm^{-1} : 3326 (NH), 1745 (ester), 1626 (amide I), 1574 (amide II), 1533 (amide II), 1435.

nmr : δ (400 MHz, CDCl_3): 1.26 (3H, d, $J=6.5$ Hz, Thr CH_3), 1.76-2.38 (8H, m, Pro $\text{C}^\beta\text{H}_2 \times 2$ + Pro $\text{C}^\gamma\text{H}_2 \times 2$), 3.40-3.94 (7H, s+m, COOCH_3 + Pro $\text{C}^\delta\text{H}_2 \times 2$), 4.18 (1H, m, Thr C^βH), 4.52 (1H, m, Thr C^αH), 4.72 (2H, m, Pro $\text{C}^\alpha\text{H}_2$), 7.26-7.64 (6H, m, Thr NH + aromatic protons).

ms : m/z : 432 (MH) $^+$.

anal: Found: C, 61.67; H, 7.03; N, 9.93 %

Calc. for $\text{C}_{22}\text{H}_{29}\text{N}_3\text{O}_6$: C, 61.25; H, 6.73; N, 9.74 %

The interesting pentapeptide Bz-Ala-Ala-Thr-Ala-Ala-OMe (122), where the Thr residue is placed at the symmetrical site, was prepared by the azide coupling route from Bz-Ala-Ala-Thr-NHNH $_2$ and H-Ala-Ala-OMe as shown in Chart C.8.c.

Bz-Ala-Ala-Thr-Ala-Ala-OMe (122): (43%)

mp. : 253-254 $^\circ\text{C}$

ir : ν_{\max} (KBr) cm^{-1} : 3271 (NH), 3071, 1753 (ester), 1690, 1627 (amide I), 1531 (amide II).

nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 0.90-1.56 (15H, m, Thr CH_3 + Ala $\text{CH}_3 \times 4$), 3.71 (3H, s, COOCH_3), 4.09-4.87 (6H, m, Thr C^αH + Thr C^βH + Ala $\text{C}^\alpha\text{H} \times 4$), 7.28-8.47 (10H, m, Thr NH + Ala NH $\times 4$ + aromatic protons).

ms : m/z: 522 (MH) $^+$.

anal: Found: C, 55.11; H, 6.49; N, 13.74 %

Calc. for $\text{C}_{24}\text{H}_{35}\text{N}_5\text{O}_8$: C, 55.28; H, 6.72; N, 13.44 %

$[\alpha]_D^{26}$: -43.13 (c, 1.66, MeOH).

The foregoing account outlined procedures used in the preparation of 70 Ser/Thr containing substrates. The reaction of these with *in situ* generated Ru(VIII) species (*vide infra*) enabled the delineation of novel and subtle aspects associated with the C^α -C side chain scission (Scheme C.2).

Chart C.9.a would show the successful chemical simulation of the PAM action with 17 substrates having at the C-terminal location, serine methyl esters, in place of glycine in the biological system. Thus, the di and tripeptide substrates shown in Chart C.9.a on treatment with Ru(VIII) species generated in a catalytic cycle from RuCl_3 (2 mole%) and NaIO_4 (18 equivalents) in $\text{CH}_3\text{CN}:\text{CCl}_4:\text{pH } 3 \text{ phosphate buffer} :: 1:1:2$ (v/v/v) at rt for 1.5 h afforded in excellent yields the terminal amides (Chart C.9.a).

Bz-NH $_2$ (2): (84%)

mp. : 126 $^\circ\text{C}$ (lit.¹⁶² mp. 128-129 $^\circ\text{C}$)

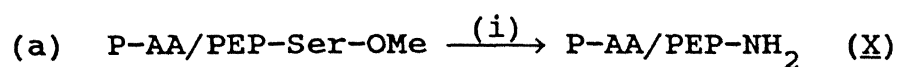
o-NO $_2$ Bz-NH $_2$ (4): (83%)

mp. : 173-174 $^\circ\text{C}$ (lit.¹⁶⁷ mp. 174-178 $^\circ\text{C}$)

ir : ν_{\max} (KBr) cm^{-1} : 3363 (NH), 3176 (NH), 1656 (amide I), 1624 (amide I), 1575, 1524 (amide II, NO $_2$), 1352 (NO $_2$).

nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 6.36-7.33 (2H, br, CONH $_2$), 7.60-8.23 (4H, m, aromatic protons).

CHART C.9



AA = Amino Acid; PEP = Peptide

P	AA/PEP (No.)	(<u>X</u>)
Bz	NIL (<u>1</u>)	Bz-NH ₂ (<u>2</u>)
o-NO ₂ Bz	NIL (<u>3</u>)	o-NO ₂ -Bz-NH ₂ (<u>4</u>)
Bz	Gly (<u>7</u>)	Bz-Gly-NH ₂ (<u>8</u>)
Bz	Ala (<u>9</u>)	Bz-Ala-NH ₂ (<u>10</u>)
Bz	Leu (<u>11</u>)	Bz-Leu-NH ₂ (<u>12</u>)
Bz	Phe (<u>13</u>)	Bz-Phe-NH ₂ (<u>14</u>)
Boc	Asp(β-OBzl) (<u>17</u>)	Boc-Asp(β-OBzl)-NH ₂ (<u>18</u>)
Bz	Glu(γ-OMe) (<u>19</u>)	Bz-Glu(γ-OMe)-NH ₂ (<u>20</u>)
Z	Asn (<u>21</u>)	Z-Asn-NH ₂ (<u>22</u>)
Z	Gln (<u>23</u>)	Z-Gln-NH ₂ (<u>24</u>)
Z	Met (<u>25</u>)	Z-Met(SO ₂)-NH ₂ (<u>26</u>)
Bz	Val (<u>27</u>)	Bz-Val-NH ₂ (<u>28</u>)
Bz	Pro (<u>29</u>)	Bz-Pro-NH ₂ (<u>30</u>)
Boc	Arg(N ^G NO ₂) (<u>31</u>)	Boc-Arg(N ^G NO ₂)-NH ₂ (<u>32</u>)
Bz	Pro-Phe (<u>33</u>)	Bz-Pro-Phe-NH ₂ (<u>34</u>)
Boc	Ala-Ala (<u>36</u>)	Boc-Ala-Ala-NH ₂ (<u>37</u>)
Bz	Val-Phe (<u>38</u>)	Bz-Val-Phe-NH ₂ (<u>39</u>)

(i) NaIO₄/RuCl₃·3H₂O/MeCN:CCl₄:pH 3 buffer/1.5 h

CHART C.9 (CONTINUED)

(b) P-AA/PEP-Ser-OMe $\xrightarrow{(i)}$ P-AA/PEP-NH-CO-COOMe (X)

P	AA/PEP (NO.)	(<u>X</u>)
Z	Gly (<u>5</u>)	Z-Gly-NH-CO-COOMe (<u>6</u>)
Bz	Asp(β -OMe) (<u>15</u>)	Bz-Asp(β -OMe)-NH-CO-COOMe (<u>16</u>)

(i) NaIO₄/RuCl₃·3H₂O/MeCN:CCl₄:pH 3 buffer/1.5 h

CHART C.10

P-AA/PEP-Thr-OMe $\xrightarrow{(i)}$ P-AA/PEP-NH-CO-COOMe (X)

AA = Amino Acid; PEP = Peptide

P	AA/PEP (No.)	(<u>X</u>)
Bz	NIL (<u>40</u>)	Bz-NH ₂ (<u>2</u>)
Bz	Gly (<u>41</u>)	Bz-Gly-NH ₂ (<u>8</u>)
Bz	Ala (<u>42</u>)	Bz-Ala-NH ₂ (<u>10</u>)
Bz	Leu (<u>43</u>)	Bz-Leu-NH ₂ (<u>12</u>)
Bz	Phe (<u>44</u>)	Bz-Phe-NH ₂ (<u>14</u>)
Bz	Gly-Phe (<u>45</u>)	Bz-Gly-Phe-NH ₂ (<u>46</u>)
Bz	Val-Phe (<u>47</u>)	Bz-Val-Phe-NH ₂ (<u>39</u>)
Boc	Ala-Ala (<u>48</u>)	Boc-Ala-Ala-NH ₂ (<u>37</u>)

(i) NaIO₄/RuCl₃·3H₂O/MeCN:CCl₄:pH 3 buffer/1.5 h

ms : m/z: 167 (MH)⁺, 166 (M)⁺.

Bz-Gly-NH₂ (9): (54%)

mp. : 170-171°C

ir : ν_{\max} (KBr) cm⁻¹: 3270 (NH), 3150 (NH), 3075, 1695 (amide I), 1675 (amide I), 1633 (amide I), 1603, 1575 (amide II), 1550 (amide II).

nmr : δ [CDCl₃ + (CD₃)₂SO]: 3.91 (2H, d, J=6.5 Hz, Gly CH₂), 6.65-8.25 (7H, m, CONH₂ + aromatic protons), 8.59 (1H, t, Gly NH).

Bz-Ala-NH₂ (10): (49%)

mp. : 232-234°C

ir : ν_{\max} (KBr) cm⁻¹: 3300 (NH), 3165 (NH), 1690 (amide I), 1635 (amide I), 1603, 1577 (amide II), 1548 (amide II).

anal: Found: C, 62.93; H, 6.16; N, 14.82 %

Calc. for C₁₀H₁₂N₂O₂: C, 62.50; H, 6.25; N, 14.58 %

$[\alpha]_D^{30}$: +21.1 (c, 1.7, MeOH).

Bz-Leu-NH₂ (12): (68%)

mp. : 169-170°C

ir : ν_{\max} (KBr) cm⁻¹: 3390 (NH), 3325 (NH), 3195 (NH), 1635, (amide I), 1612 (amide I), 1588, 1562 (amide II), 1532 (amide II).

nmr : δ [CDCl₃ + (CD₃)₂SO]: 0.91 (6H, d, J=5.0 Hz, Leu CH₃×2), 1.69 (3H, m, Leu C ^{β} H₂ + Leu C ^{γ} H), 4.75 (1H, m, Leu C ^{α} H), 5.60-6.82 (2H, br, CONH₂), 6.96 (1H, d, J=7.5 Hz, Leu NH), 7.34-8.00 (5H, m, aromatic protons).

anal: Found: C, 67.08; H, 8.11; N, 11.77 %

Calc. for C₁₃H₁₈N₂O₂: C, 66.67; H, 7.69; N, 11.97 %

$[\alpha]_D^{30}$: +2.1 (c, 1.6, CHCl₃).

* Reaction of Bz-Leu-Ser-OMe (11) with Ru(VIII) at pH 6 for 1.5 h also afforded Bz-Leu-NH₂ (12) in 30% yields.

Bz-Phe-NH₂ (14): (79%)

mp. : 183-184°C

ir : ν_{\max} (KBr)cm⁻¹: 3410, 3335 (NH), 3200 (NH), 1662 (amide I),
1635 (amide I), 1608, 1583 (amide II), 1525 (amide II).

anal: Found: C, 71.40; H, 6.22; N, 10.54 %

Calc. for C₁₆H₁₆N₂O₂: C, 71.64; H, 5.97; N, 10.45 %

$[\alpha]_D^{30}$: -27.8 (c, 2.8, MeOH).

Boc-Asp(β -OBzl)-NH₂ (18): (96%)

mp. : 145-146°C

ir : ν_{\max} (KBr)cm⁻¹: 3405 (NH), 3350 (NH), 3210 (NH), 1725 (Bzl
ester), 1665 (amide I), 1635 (amide I), 1510 (amide II).

nmr : δ (CDCl₃): 1.43 (9H, s, Boc CH₃×3), 2.87 (2H, m, Asp
C ^{β} H₂), 4.53 (1H, m, Asp C ^{α} H), 5.12 (2H, s, Bzl CH₂), 5.65
(1H, br, Asp NH), 6.40 (2H, br, CONH₂), 7.37 (5H, s,
aromatic protons).

ms : m/z: 323 (MH)⁺.

anal: Found: C, 59.36; H, 6.63; N, 8.53 %

Calc. for C₁₆H₂₂N₂O₅: C, 59.63; H, 6.83; N, 8.70 %

$[\alpha]_D^{30}$: +33.83 (c, 0.13, CHCl₃).

Bz-Glu(γ -OMe)-NH₂ (20): (90%)

mp. : 136-137°C

ir : ν_{\max} (KBr)cm⁻¹: 3396 (NH), 3306 (NH), 3185 (NH), 1730
(ester), 1659 (amide I), 1633 (amide I), 1577 (amide II),
1523 (amide II).

nmr : δ [CDCl₃ + (CD₃)₂SO]: 1.87-2.56 (4H, m, Glu C ^{β} H₂ + Glu
C ^{γ} H₂), 3.69 (3H, s, COOCH₃), 4.59 (1H, m, Glu C ^{α} H),
7.06-8.03 (8H, m, Glu NH + CONH₂ + aromatic protons).

ms : m/z: 264 (M)⁺.

anal: Found: C, 58.69; H, 5.76; N, 10.64 %

Calc. for C₁₃H₁₆N₂O₄: C, 59.09; H, 6.06; N, 10.61 %

Z-Asn-NH₂ (22): (65%)

mp. : 220-222°C (lit.¹⁶⁴ mp. 225-226°C)

ir : ν_{\max} (KBr) cm⁻¹: 3383 (NH), 3321 (NH), 3185 (NH), 1696 (amide I), 1655 (amide I), 1533 (amide II).

nmr : δ [CDCl₃ + (CD₃)₂SO]: 2.50 (2H, d, J=6.5 Hz, Asn C ^{β} H₂), 4.34 (1H, m, Asn C ^{α} H), 5.07 (2H, s, Z CH₂), 6.73-7.31 (10H, s+m, Asn NH + CONH₂×2 + aromatic protons).

ms : m/z: 266 (MH)⁺.

anal: Found: C, 54.25; H, 5.36; N, 15.75 %

Calc. for C₁₂H₁₅N₃O₄: C, 54.34; H, 5.66; N, 15.85 %

[α]_D³⁰: -2.32 (c, 0.56, MeOH).

Z-Gln-NH₂ (24): (90%)

mp. : 138-139°C

ir : ν_{\max} (KBr) cm⁻¹: 3390 (NH), 3315 (NH), 3199 (NH), 1654 (br, amide I), 1539 (amide II).

nmr : δ [CDCl₃ + (CD₃)₂SO]: 1.50-2.31 (4H, m, Gln C ^{β} H₂ + Gln C ^{γ} H₂), 3.96 (1H, m, Gln C ^{α} H), 5.09 (2H, s, Z CH₂), 6.64-7.50 (10H, s+m, Gln NH + CONH₂×2 + aromatic protons).

ms : m/z: 280 (MH)⁺.

anal: Found: C, 56.22; H, 6.04; N, 14.83 %

Calc. for C₁₃H₁₇N₃O₄: C, 55.91; H, 6.09; N, 15.05 %

[α]_D³⁰: +3.20 (c, 0.25, MeOH).

Z-Met(SO)₂-NH₂ (26): (95%)

mp. : 111-112°C

ir : ν_{\max} (KBr) cm⁻¹: 3425 (NH), 3380 (NH), 3190 (NH), 1650 (amide I), 1525 (amide II).

nmr : δ [CDCl₃ + (CD₃)₂SO]: 2.00-3.34 (7H, s + m, COOCH₃ + Met C ^{β} H₂ + Met C ^{γ} H₂), 4.37 (1H, m, Met C ^{α} H), 5.12 (2H, s, Z CH₂), 6.62 (1H, brd, Met NH), 7.40 (7H, s + m, CONH₂ + aromatic protons)

ms : m/z: 315 (MH)⁺.

anal: Found: C, 49.43; H, 5.94; N, 8.63 %

Calc. for C₁₃H₁₈N₂O₅S: C, 49.68; H, 5.73; N, 8.92 %

[α]_D³⁰: +5.65 (c, 0.56, MeOH).

Bz-Val-NH₂ (28): (95%)

mp. : 216-217°C

ir : ν_{max}(KBr)cm⁻¹: 3400 (NH), 3320 (NH), 3210 (NH), 1660 (amide I), 1632 (amide I), 1602, 1578 (amide II), 1520 (amide II).

nmr : δ [CDCl₃ + (CD₃)₂SO]: 1.00 (6H, dd, J=5.0 Hz, 2.5 Hz, Val CH₃x2), 2.15 (1H, m, Val C^βH), 4.50 (1H, m, Val C^αH), 7.12-7.96 (8H, m, Val NH + CONH₂ + aromatic protons).

ms : m/z: 221 (MH)⁺.

anal: Found: C, 65.62; H, 7.44; N, 12.84 %

Calc. for C₁₂H₁₆N₂O₂: C, 65.46; H, 7.27; N, 12.73 %

Bz-Pro-NH₂ (30): (86%)

mp. : syrup

ir : ν_{max}(KBr)cm⁻¹: 3370 (NH), 3180 (NH), 1660 (amide I), 1602, 1562 (amide II).

nmr : δ [CDCl₃ + (CD₃)₂SO]: 2.12 (4H, m, Pro C^βH₂ + Pro C^γH₂), 3.59 (2H, m, Pro C^δH₂), 4.71 (1H, m, Pro C^αH), 7.46 (7H, m, CONH₂ + aromatic protons).

ms : m/z: 219 (MH)⁺.

anal: Found: C, 66.18; H, 6.52; N, 12.93 %

Calc. for C₁₂H₁₄N₂O₂: C, 66.06; H, 6.42; N, 12.84 %

[α]_D³⁰: -56.85 (c, 0.35, MeOH).

Boc-Arg(N^GNO₂)-NH₂ (32): (91%)

mp. : syrup

ir : ν_{max}(KBr)cm⁻¹: 3324 (br, NH), 1675 (br, amide I), 1625 (amide I), 1595, 1527 (NO₂), 1367 (NO₂).

nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 1.40 (9H, s, Boc $\text{CH}_3 \times 3$), 2.00 (4H, m, Arg C^βH_2 + Arg $\text{C}^\gamma\text{H}_2$), 3.34 (2H, m, Arg $\text{C}^\delta\text{H}_2$), 4.06 (1H, m, Arg C^αH), 5.53 (1H, br, Arg NH), 7.00-7.56 (5H, br, CONH_2 + guanidino $\text{NH} \times 3$).

ms : m/z : 319 $(\text{MH})^+$.

anal: Found: C, 41.09; H, 7.23; N, 27.11 %

Calc. for $\text{C}_{11}\text{H}_{22}\text{N}_6\text{O}_5$: C, 41.51; H, 6.92; N, 26.41 %

Bz-Pro-Phe- NH_2 (34): (70%)

mp. : 188-190°C

ir : ν_{max} (KBr) cm^{-1} : 3310 (NH), 3160 (NH), 1675 (amide I), 1655 (amide I), 1615, 1532 (amide II).

nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 1.50-2.33 (4H, m, Pro C^βH_2 + Pro $\text{C}^\gamma\text{H}_2$), 2.90-3.80 (4H, m, Pro $\text{C}^\delta\text{H}_2$ + Phe C^βH_2), 4.40-4.75 (2H, m, Pro C^αH + Phe C^αH), 6.70 (1H, brs, Phe NH), 7.00-8.00 (7H, s + m, CONH_2 + aromatic protons).

anal: Found: C, 69.22; H, 6.27; N, 11.64 %

Calc. for $\text{C}_{21}\text{H}_{23}\text{N}_3\text{O}_3$: C, 69.04; H, 6.30; N, 11.51 %

$[\alpha]_D^{30}$: -75.2 (c, 2.0, MeOH).

Boc-Ala-Ala- NH_2 (37): (78%)

mp. : 158-159°C (lit.¹⁶⁵ mp. 162-163°C)

ir : ν_{max} (KBr) cm^{-1} : 3390 (NH), 3350 (NH), 3315 (NH), 3205 (NH), 1685 (amide I), 1645 (amide I), 1540 (amide II).

nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 1.26 (3H, d, $J=6.5$ Hz, Ala CH_3), 1.37 (3H, d, $J=6.5$ Hz, Ala CH_3), 1.43 (9H, s, Boc $\text{CH}_3 \times 3$), 3.87-4.59 (2H, m, Ala $\text{C}^\alpha\text{H} \times 2$), 6.10-7.20 (3H, m, Ala NH(Boc) + CONH_2), 7.53 (1H, d, $J=7.5$ Hz, Ala NH).

anal: Found: C, 50.84; H, 7.93; N, 16.64 %

Calc. for $\text{C}_{11}\text{H}_{21}\text{N}_3\text{O}_4$: C, 50.97; H, 8.11; N, 16.22 %

$[\alpha]_D^{30}$: -38.9 (c, 1.7, MeOH).

Bz-Val-Phe-NH₂ (39): (65%)

mp. : 238-239°C

ir : ν_{\max} (KBr) cm⁻¹: 3423 (NH), 3318 (NH), 3275 (NH), 3215 (NH),
1675 (amide I), 1655 (amide I), 1632 (amide I), 1618, 1580
(amide II), 1540 (amide II).

anal: Found: C, 68.38; H, 7.16; N, 11.29 %

Calc. for C₂₁H₂₅N₃O₃: C, 68.66; H, 6.81; N, 11.44 %

$[\alpha]_D^{30}$: -26.8 (c, 0.9, MeOH).

The same type of reaction profile can be seen in Chart C.10 wherein a set of 8 dipeptides and tripeptides having C-terminal Thr esters smoothly afforded, under the above conditions, in excellent yields, the expected terminal amides.

Bz-Gly-Phe-NH₂ (46): (51%)

mp. : 177-178°C

ir : ν_{\max} (KBr) cm⁻¹: 3435 (NH), 3380 (NH), 3285 (NH), 3210 (NH),
1682 (amide I), 1670 (amide I), 1640 (amide I), 1607, 1580
(amide II), 1540 (amide II), 1492.

nmr : δ [CDCl₃ + (CD₃)₂SO]: 3.03 (2H, m, Phe C ^{β} H₂), 3.87 (2H, m,
Gly CH₂), 4.60 (1H, m, Phe C ^{α} H), 6.60-8.03 (13H, s+m, Phe
NH + CONH₂ + aromatic protons), 8.50 (1H, t, Gly NH).

anal: Found: C, 66.11; H, 5.75; N, 12.74 %

Calc. for C₁₈H₁₉N₃O₃: C, 66.46; H, 5.85; N, 12.92 %

$[\alpha]_D^{30}$: +2.6 (c, 2.6, MeOH).

The facile C-N bond rupture of Ser/Thr residues is rationalized on the basis of fragmentation of a carbinolamide arising from addition of water to the initially formed acylimine, which in turn, is produced by the oxidative scission of a Ser/Thr C ^{α} -C side chain bond, involving either a cyclic or an open chain ruthenium intermediate (Scheme C.2). The overall process generates a C-terminal amide retaining the Ser/Thr nitrogen atom and releasing the C₂ unit of carbinolamide as glyoxylate.

The oxidative scission of Ser/Thr extended precursors to des-Ser/Thr peptide amides¹⁷⁶ simulates the natural process of terminal α -amidation in the post-translational processing of Gly extended hormonal precursors by pituitary enzymes. It is important to note that both the natural as well as the chemical processes proceed with the intermediacy of a carbinolamide. The parallel between the strategies is shown in Scheme C.3.

Noteworthy features of the terminal amidation mediated by Ru(VIII) species and presented in Chart C.9.a and C.10 are, the stability of diverse N-protecting groups, the total unreactivity of the potentially susceptible side chains of Phe, Asn, Gln, Pro and nitro-arginine and the clean transformation of the methionine side chain to the sulfone.

All the products in this and subsequent studies have been fully characterized and chiral retention in the process established.

The isolation of crystalline, chain intact, oxalamido ester in the oxidation of Z-Gly-Ser-OMe (5) and Bz-Asp(β -OMe)-Ser-OMe (15) (Chart C.9.b) brought to light subtle facets pertaining to this reaction. Further

Z-Gly-NH-CO-CO₂Me (6): (90%)

mp. : 92-93°C

ir : ν_{\max} (KBr)cm⁻¹: 3370 (NH), 3260 (NH), 3190 (NH), 1742 (ester), 1678 (carbamate), 1525 (amide II), 1490.

nmr : δ [CDCl₃ + (CD₃)₂SO]: 3.88 (3H, s, COOCH₃), 4.09 (2H, d, J=6.5 Hz, Gly CH₂), 5.13 (2H, s, Z CH₂), 7.06 (1H, br, exchangeable with D₂O, Gly NH), 7.38 (5H, s, aromatic protons), 11.25 (1H, s, exchangeable, CONHCO).

ms : m/z: 295 (MH)⁺.

anal: Found: C, 53.21; H, 4.47; N, 9.39 %

Calc. for C₁₃H₁₄N₂O₆: C, 53.06; H, 4.76; N, 9.52 %

Bz-Asp(β -OMe)-NH-CO-CO₂Me (16): (92%)

mp. : 156-157°C

ir : ν_{\max} (KBr)cm⁻¹: 3271 (NH), 1782 (CONHCO), 1734 (ester),

1633 (amide I), 1578 (amide II), 1531 (amide II), 1500.

nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 2.93 (2H, m, Asp C^βH_2), 3.68, 3.87 (3H, 3H, s, s, $\text{COOCH}_3 \times 2$), 5.03 (1H, m, Asp C^αH), 7.25-8.06 (5H, m, aromatic protons), 8.65 (1H, d, $J=7.5$ Hz, Asp NH), 11.40 (1H, s, CONHCO).

ms : m/z : 337 (MH)⁺.

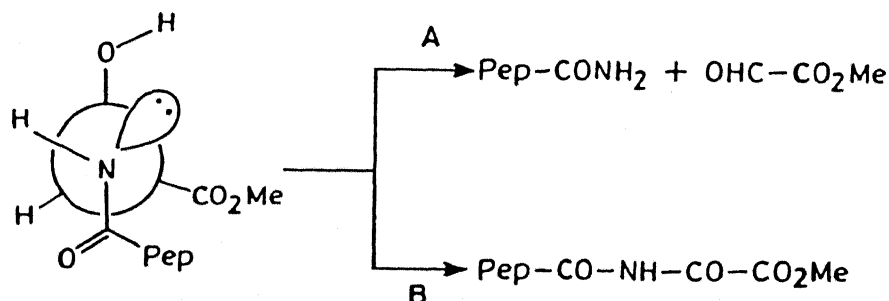
anal: Found: C, 53.73; H, 4.48; N, 8.26 %

Calc. for $\text{C}_{15}\text{H}_{16}\text{N}_2\text{O}_7$: C, 53.57; H, 4.76; N, 8.33 %

$[\alpha]_D^{30}$: -26.87 (c, 0.64, MeOH).

work (*vide infra*) has shown that the pH of the medium, duration of the reaction and the nature of substrates involved can play a role in the reaction course. The oxalamides naturally arise from further oxidation of the initially formed carbinolamides. Thus, two options are available to carbinolamides for further reaction, namely, N-C bond scission to terminal amides or further oxidation to oxalamides (Scheme C.4).

Scheme C.4



All evidence points that wherever the anti-periplanarity, necessary for path A, is difficult, further oxidation via pathway B becomes dominant. The fact that when Pep is $\text{Bz-NH-CH}(\text{CH}_2\text{CO}_2\text{Me})-$, the preferred pathway is B and that in all other cases when Pep is $\text{Z-NH-CH(R)}-$, pathway A dominates, clearly shows that the amino acid side chains generally promote pathway A. Further, the experimental finding that under the same conditions when $\text{R} = -\text{CH}_2\text{COOMe}$ (**15**), path B is followed and that when $\text{R} = \text{CH}_2-\text{COOCH}_2\text{Ph}$ (**17**), path A is preferred, both exclusively, show that the controls here are

very subtle indeed!

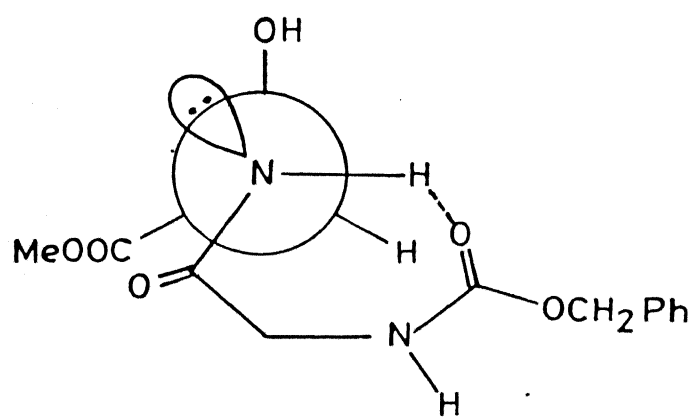
This aspect is further reinforced with the observation that both Bz-Gly-Ser-OMe (7) and Bz-Gly-Thr-OMe (41) underwent normal C-terminal amidation (Chart C.9.a and C.10).

In Scheme C.4 is illustrated the fact that both terminal amidation, involving N-C bond scission [Path A], and oxalamide formation, involving the oxidation of secondary hydroxyl group [Path B], have a common carbinolamine intermediate. It is obvious from examination of Chart C.9 and Chart C.10 that subtle factors can tilt one path in favour of the other. Clearly, in order that terminal amidation may take place, it is necessary that a transition state wherein the C-OH and the N-CO bonds are anti-periplanar. Whenever this configuration is disfavoured the oxidation pathway (Scheme C.4.B) would predominate.

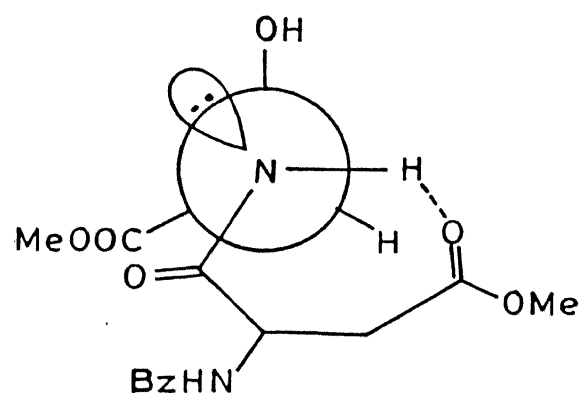
This concept has enabled the rationalization of abnormal results obtained with Bz-Asp(β -OMe)-Ser-OMe (15) and Z-Gly-Ser-OMe (5). As shown in Scheme C.5 an "in face" seven membered hydrogen bonding involving the carbinolamide NH and a side chain carbonyl would necessarily impede the required anti-periplanar transition state and would thus promote oxidation. Such a transition profile is envisaged for Z-Gly-Ser-OMe (5) (Scheme C.5.A) and Bz-Asp(β -OMe)-Ser-OMe (15) (Scheme C.5.B).

Molecular modelling studies brought out the very interesting fact that whereas a seven membered hydrogen bonded preferred conformation is not possible in the case of Ser/Thr methyl esters, the same residues when located at the N-terminal or non-terminal locations would have the option for such a transition state (Scheme C.5.C), one that would promote oxalamide formation. Even more interesting was the rationalization that where the C-terminal Ser/Thr esters be replaced by the corresponding amides, a similar hydrogen bonded transition state for the carbinolamide becomes feasible (Scheme C.5.D), thus leading to the possibility that these substrates would lead to oxalamides rather than undergoing terminal

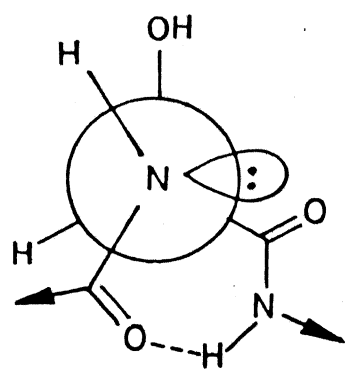
Scheme C.5



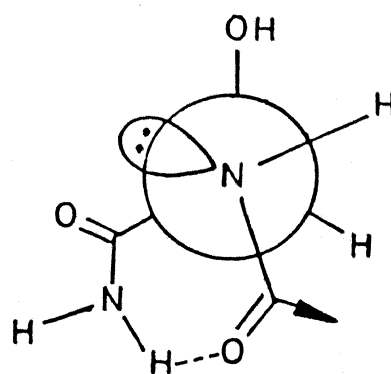
(A)



(B)



(C)



(D)

amidation, as has been established with the esters.

Thus, possibilities for alternate pathways, leading to oxalamides, were anticipated in Ru(VIII) mediated C^α-C side chain scission of Ser/Thr residues located at the N-terminal or non-terminal locations on the one hand and for the substrates having Ser/Thr-NH₂ termini on the other. In the event, these expectations were fully realized leading to the delineation of novel methodologies for the placement of oxalamide units in the peptide backbone (*vide supra*).

Chart C.11.a illustrates the transformation of 8 dipeptides of the profile P-Ser-AA-OMe having the serine residue at the N-terminal end. Precisely under conditions described previously, these were smoothly transformed to the corresponding oxalamides in excellent yields, thus supporting the rationalization of a transition state similar to that shown in Scheme C.5.C.

Z-NH-CO-CO-Gly-OMe (50): (91%)

mp. : 132-133°C

ir : ν_{\max} (KBr)cm⁻¹: 3250 (NH), 3215 (NH), 3185 (NH), 3050, 1778 (CONHCO), 1755 (carbonyl), 1745 (ester), 1698 (carbamate), 1680 (amide I), 1500 (amide II).

nmr : δ (CDCl₃): 3.81 (3H, s, COOCH₃), 4.11 (2H, d, J=6.5 Hz, Gly CH₂), 5.25 (2H, s, Z CH₂), 7.40 (5H, s, aromatic protons), 7.81 (1H, br, exchangeable with D₂O, Gly NH), 9.37 (1H, brs, exchangeable, CONHCO).

¹³C nmr : δ (100 MHz, CDCl₃): 41.4 (Gly CH₂), 52.6 (OCH₃), 68.4 (OCH₂), 128.5, 128.7, 134.5 (Ph), 149.7 (Z CO), 157.0, 158.3 (-COCO-), 168.5 (Gly-CO).

ms : m/z: 294 (M)⁺.

anal: Found: C, 52.78; H, 4.63; N, 9.26 %

Calc. for C₁₃H₁₄N₂O₆: C, 53.06; H, 4.76; N, 9.52 %

Z-NH-CO-CO-Ala-OMe (52): (66%)

mp. : 70-71°C

ir : ν_{\max} (KBr) cm^{-1} : 3332 (NH), 3254 (NH), 1792 (CONHCO carbonyl), 1749 (ester), 1691 (amide I), 1478 (br, amide II).

nmr : δ (CDCl_3): 1.46 (3H, d, $J=6.5$ Hz, Ala CH_3), 3.75 (3H, s, COOCH_3), 4.53 (1H, m, Ala C^αH), 5.22 (2H, s, Z CH_2), 7.34 (5H, s, aromatic protons), 7.87 (1H, brd, exchangeable with D_2O , Ala NH), 9.43 (1H, brs, exchangeable, CONHCO).

^{13}C nmr : δ (100 MHz, CDCl_3): 17.8 (Ala CH_3), 48.7 (OCH_3), 52.7 (Ala C^αH), 68.3 (OCH_2), 128.5, 128.7, 134.6 (Ph), 149.7 (Z CO), 157.1, 157.5 ($-\text{COCO}-$), 171.5 (Ala-CO).

ms : m/z : 309 (MH)⁺.

anal: Found: C, 54.74; H, 5.46; N, 8.78 %

Calc. for $\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}_6$: C, 54.55; H, 5.19; N, 9.09 %Z-NH-CO-CO-Phe-OMe (54): (82%)

mp. : 82-83°C

ir : ν_{\max} (KBr) cm^{-1} : 3318 (NH), 1779 (CONHCO), 1740 (ester), 1718 (carbamate), 1677 (amide I), 1474 (br, amide II).

nmr : δ (60 MHz, CDCl_3): 3.15 (2H, d, $J=6.0$ Hz, Phe C^βH_2), 3.70 (3H, s, COOCH_3), 4.76 (1H, m, Phe C^αH), 5.18 (2H, s, Z CH_2), 6.09-7.50 (10H, s + m, aromatic protons), 7.73 (1H, brd, exchangeable with D_2O , Phe NH), 9.26 (1H, brs, exchangeable, CONHCO).

anal: Found: C, 62.17; H, 5.05; N, 6.93 %

Calc. for $\text{C}_{20}\text{H}_{20}\text{N}_2\text{O}_6$: C, 62.50; H, 5.21; N, 7.29 % $[\alpha]_D^{25}$: +32.0 (c, 3.2, CHCl_3).Z-NH-CO-CO-Leu-OMe (56): (85%)

mp. : syrup

ir : ν_{\max} (neat) cm^{-1} : 3321 (br, NH), 1793 (CONHCO carbonyl), 1743 (ester), 1690 (br, amide I), 1487 (br, amide II).

nmr : δ (CDCl_3): 0.87 (6H, d, $J=5.0$ Hz, Leu $\text{CH}_3 \times 2$), 1.62 (3H, m, Leu C^βH_2 + Leu C^γH), 3.71 (3H, s, COOCH_3), 4.53 (1H, m, Leu C^αH), 5.20 (2H, s, Z CH_2), 7.34 (5H, s, aromatic protons), 7.75 (1H, d, $J=7.5$ Hz, Leu NH), 9.43 (1H, brs, CONHCO).

ms : m/z : 350 (M)⁺.

anal: Found: C, 58.73; H, 6.48; N, 8.36 %

Calc. for $\text{C}_{17}\text{H}_{22}\text{N}_2\text{O}_6$: C, 58.29; H, 6.29; N, 8.00 %

$[\alpha]_D^{25}$: -7.5 (c, 13.5, CHCl_3).

Z-NH-CO-CO-Methylantranilate (58): (96%)

mp. : 125-126°C

ir : ν_{\max} (KBr) cm^{-1} : 3302 (NH), 3185 (NH), 1758 (CONHCO carbonyl), 1735 (ester), 1702 (carbamate), 1687 (amide I), 1603, 1588, 1541 (amide II), 1491.

nmr : δ (60 MHz, CDCl_3): 3.93 (3H, s, COOCH_3), 5.26 (2H, s, Z CH_2), 6.93-7.76 (7H, s + m, anthranilic ring proton $\times 2$ + Z-aromatic protons), 8.00 (2H, m, anthranilic NH + anthranilic ring proton), 8.56 (1H, d, $J=9.0$ Hz, anthranilic ring proton), 9.41 (1H, s, exchangeable with D_2O , CONHCO).

ms : m/z : 356 (M)⁺.

anal: Found: C, 60.89; H, 4.34; N, 7.64 %

Calc. for $\text{C}_{18}\text{H}_{16}\text{N}_2\text{O}_6$: C, 60.67; H, 4.49; N, 7.87 %

Z-NH-CO-CO-Pro-OMe (63): (50%)

mp. : syrup

ir : ν_{\max} (neat) cm^{-1} : 3360 (NH), 3278 (NH), 1790 (CONHCO), 1734 (br, ester), 1652 (amide I), 1560 (amide II).

nmr : δ (CDCl_3): 2.09 (4H, m, Pro C^βH_2 + Pro $\text{C}^\gamma\text{H}_2$), 3.81 (3H, s, COOCH_3), 4.03 (2H, m, Pro $\text{C}^\delta\text{H}_2$), 4.60 (1H, m, Pro C^αH),

5.26 (2H, s, Z CH₂), 7.47 (5H, s, aromatic protons), 9.75

(1H, brs, CONHCO).

ms : m/z: 335 (MH)⁺.

anal: Found: C, 57.76; H, 5.43; N, 8.44 %

Calc. for C₁₆H₁₈N₂O₆: C, 57.49; H, 5.39; N, 8.38 %

Z-NH-CO-CO-Asp(β-OMe)-OMe (65): (85%)

mp. : syrup

ir : ν_{\max} (neat)cm⁻¹: 3333 (NH), 1788 (CONHCO), 1729 (ester),
1687 (br, amide I), 1485 (br, amide II).

nmr : δ (60 MHz, CDCl₃): 2.96 (2H, m, Asp C^βH₂), 3.68, 3.76 (3H,
3H, s, s, COOCH₃x2), 4.80 (1H, m, Asp C^αH), 5.23 (2H, s, Z
CH₂), 7.30 (5H, s, aromatic protons), 8.06 (1H, brd,
exchangeable with D₂O, Asp NH), 9.30 (1H, s, exchangeable,
CONHCO).

ms : m/z: 367 (MH)⁺.

anal: Found: C, 52.43; H, 4.67; N, 7.56 %

Calc. for C₁₆H₁₈N₂O₈: C, 52.46; H, 4.92; N, 7.65 %

Z-NH-CO-CO-Met(SO₂)-OMe (68): (88%)

mp. : 180-181°C

ir : ν_{\max} (KBr)cm⁻¹: 3300 (NH), 1785 (CONHCO), 1750 (ester),
1670 (amide I), 1497 (br, amide II), 1375, 1122 (SO₂).

nmr : δ (CDCl₃): 2.44 (2H, m, Met C^βH₂), 2.92 (3H, s, SO₂CH₃),
3.04 (2H, m, Met C^γH₂), 3.81 (3H, s, COOCH₃), 4.66 (1H, m,
Met C^αH), 5.22 (2H, s, Z CH₂), 7.35 (5H, s, aromatic
protons), 8.19 (1H, d, J=7.5 Hz, exchangeable with D₂O,
Met NH), 9.38 (1H, s, exchangeable, CONHCO).

ms : m/z: 401 (MH)⁺.

[α]_D²⁵: -14.3 (c, 1.83, MeOH).

The Ru(VIII) mediated oxidation of Z-Ser-Ser-OMe (66), in principle, afforded possibilities for the operation of diverse reaction pathways. The anticipation was that in this compound the N-terminal Ser would be converted to an oxalamide and the C-terminal Ser-OMe to the corresponding terminal amide. In the event, however, the product isolated was Z-NH-CO-CO-NH₂ (60) arising from preferential oxidation of the N-terminal serine (Chart C.11.c).

Z-NH-CO-CO-NH₂ (60): (32%)

mp. : 198-200°C

ir : ν_{\max} (KBr)cm⁻¹: 3375 (NH), 3171 (NH), 1776 (CONHCO), 1684 (br, amide I), 1515 (br, amide II).

nmr : δ [CDCl₃ + (CD₃)₂SO]: 5.25 (2H, s, Z CH₂), 7.40 (5H, s, aromatic protons), 8.00 (2H, br, exchangeable with D₂O, CONH₂), 10.31 (1H, s, exchangeable, CONHCO).

ms : m/z: 223 (MH)⁺.

Interestingly, the desired transformation of both the serine residues in the anticipated manner was realized on oxidation of three tripeptides, namely Z-Ser-Aib-Ser-OMe (69), Z-Ser-Leu-Ser-OMe (71) and Z-Ser-Gly-Ser-OMe (73). Thus, in these substrates the N-terminal Ser was transformed to oxalamide (Scheme C.4.B) and the C-terminal Ser-OMe residue underwent terminal amidation (Scheme C.4.A) leading to respectively (70), (72) and (74) in good yields (Chart C.11.d).

Z-NH-CO-CO-Aib-NH₂ (70): (84%)

mp. : syrup

ir : ν_{\max} (KBr)cm⁻¹: 3486 (NH), 3348 (NH), 1785 (CONHCO), 1687 (br, amide I), 1497 (amide II).

nmr : δ (CDCl₃): 1.62 (6H, brs, Aib CH₃x2), 5.18 (2H, s, Z CH₂), 5.96 (2H, br, CONH₂), 7.33 (5H, s, aromatic protons), 8.03 (1H, s, Aib NH), 9.40 (1H, s, CONHCO).

ms : m/z: 308 (MH)⁺.

anal: Found: C, 54.63; H, 5.88; N, 13.43 %

Calc. for C₁₄H₁₇N₃O₅: C, 54.72; H, 5.54; N, 13.68 %

Z-NH-CO-CO-Leu-NH₂ (72): (50%)

mp. : syrup

ir : ν_{\max} (neat) cm⁻¹: 3392 (NH), 3294 (NH), 3194 (NH), 1783 (CONHCO), 1659 (br, amide I), 1492 (amide II).

nmr : δ (60 MHz, CDCl₃): 0.91 (6H, brd, Leu CH₃x2), 1.63 (3H, m, Leu C ^{β} H₂ + Leu C ^{γ} H), 4.43 (1H, m, Leu C ^{α} H), 5.18 (2H, s, Z CH₂), 6.30 (2H, br, exchangeable with D₂O, CONH₂), 7.31 (5H, s, aromatic protons), 8.05 (1H, d, J=7.5 Hz, exchangeable, Leu NH), 9.50 (1H, s, exchangeable, CONHCO).

ms : m/z: 335 (M)⁺.

anal: Found: C, 57.43; H, 6.48; N, 12.17 %

Calc. for C₁₆H₂₁N₃O₅: C, 57.31; H, 6.27; N, 12.54 %

Z-NH-CO-CO-Gly-NH₂ (74): (83%)

mp. : 204-205^oc

ir : ν_{\max} (KBr) cm⁻¹: 3402 (NH), 3208 (NH), 1772 (CONHCO), 1686 (amide I), 1654 (amide I), 1493 (amide II).

nmr : δ [CDCl₃ + (CD₃)₂SO]: 3.80 (2H, d, J=6.5 Hz, Gly CH₂), 5.22 (2H, s, Z CH₂), 6.80-7.42 (7H, m, CONH₂ + aromatic protons), 9.00 (1H, t, exchangeable, Gly NH), 10.75 (1H, s, exchangeable, CONHCO).

ms : m/z: 279 (M)⁺.

anal: Found: C, 51.29; H, 4.38; N, 15.35 %

Calc. for C₁₂H₁₃N₃O₅: C, 51.61; H, 4.66; N, 15.05 %

The subtle factors that control the overall process of the Ru(VIII) mediated C ^{α} -C side chain scission of the Ser residue is again reflected in the oxidation of Z-Ser-Pro-Ser-OMe (75) which afforded Z-NH-CO-CO-Pro-NH₂

(76) arising from expected pathways and Z-Ser-Pro-NH₂ (77) wherein the C-terminal Ser alone underwent change (Chart C.11.e).

Z-NH-CO-CO-Pro-NH₂ (76): (40%)

mp. : gummy

ir : ν_{\max} (KBr) cm⁻¹: 3348 (NH), 1785 (CONHCO), 1679 (br, amide I), 1497 (amide II).

nmr : δ (CDCl₃): 2.10 (4H, m, Pro C ^{β} H₂ + Pro C ^{γ} H₂), 3.80 (2H, m, Pro C ^{γ} H₂), 4.50 (1H, m, Pro C ^{α} H), 5.20 (2H, s, Z CH₂), 7.33 (7H, brs, CONH₂ + aromatic protons), 9.80 (1H, s, CONHCO).

ms : m/z: 320 (MH)⁺.

Z-Ser-Pro-NH₂ (77): (30%)

mp. : 205-206°C

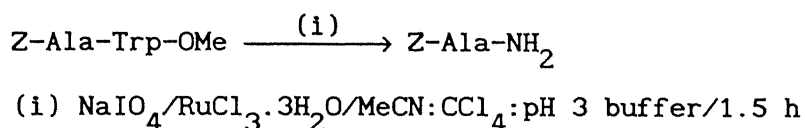
ir : ν_{\max} (KBr) cm⁻¹: 3220, 1672 (br).

nmr : δ (CDCl₃): 2.00 (4H, br, Pro C ^{β} H₂ + Pro C ^{γ} H₂), 3.31-4.03 (4H, m, Pro C ^{δ} H₂ + Ser C ^{β} H₂), 5.09 (4H, s+br, Pro C ^{α} H + Ser C ^{α} H + Z CH₂), 6.15 (1H, br, exchangeable, Ser NH), 6.93-7.53 (7H, s+br, CONH₂ + aromatic protons).

The substrates thus far subjected to Ru(VIII) oxidation and similar ones having N-terminal threonine residue (*vide infra*) afforded oxalamides in accordance with expectations based on mechanistic pathway envisaged in Scheme C.5.C. Yet another novel finding, having important implications pertaining to PAM action and protein rupture, emerged from similar oxidations with either Z-Ser-Trp-OMe (61) or Z-Ser-Tyr-OMe (59). These substrates provided a very different reaction profile. Here, not only was the Trp/Tyr side chain oxidized to that of aspartic acid (65), which was fully characterized as the ester, but also, in sum, the Trp/Tyr side chains acted as the terminal Ser/Thr residues in Ru(VIII) oxidations or as equivalent to the C-terminal Gly residues in PAM action, leading to

terminal amides, leading to the isolation of Z-NH-CO-CO-NH₂ (60) (Chart C.11.b).

The fact that Z-Ser-Asp(β -OMe)-OMe (64) (Chart C.11) gave the expected Z-NH-CO-CO-Asp(β -OMe)-OMe (65), coupled with earlier finding¹⁷⁷ that Tyr and Trp side chains are oxidized readily with Ru(VIII) to CH₂-COOH, clearly showed that in both cases this unit is responsible for the scission. This notion was confirmed by treatment of Z-Ala-Trp-OMe, under identical conditions, to afford Z-Ala-NH₂ (38%).



The formation of Z-NH-CO-CO-NH₂ (60) from either Z-Ser-Trp-OMe (61) or Z-Ser-Tyr-OMe (59) can be rationalized as proceeding by any of the three pathways illustrated in Scheme C.6. It may be noted from Scheme C.6 that pathways I and II resemble terminal amidation involving carbinolamide intermediates. Pathway III is an elimination process with implication that an aspartic acid side chain on its own can bring about terminal amidation! This possibility was ruled out since Z-Ala-Asp(β -OH)-OMe, independently prepared from Z-Ala-Asp(β -OBzl)-OMe, was found stable under the relevant reaction conditions at pH 3.

Taken together, these findings clearly support a mechanism wherein an Asp(β -OH) side chain can bring about the oxidative N-C bond scission of proximally placed oxalamide or peptide, the former, as expected, being more efficient. This novel finding can be rationalized on the basis of a cyclic or open ruthenium intermediate (Scheme C.7).

Transformation of peptides having N-terminal threonine units was clearly experimentally demonstrated with ten examples (Chart C.12.a, Chart C.12.b). As could be seen from Chart C.12, in every case, the expected N ^{α} -protected oxalamido peptides were indeed formed.

Scheme C.6

Proposed Mechanism

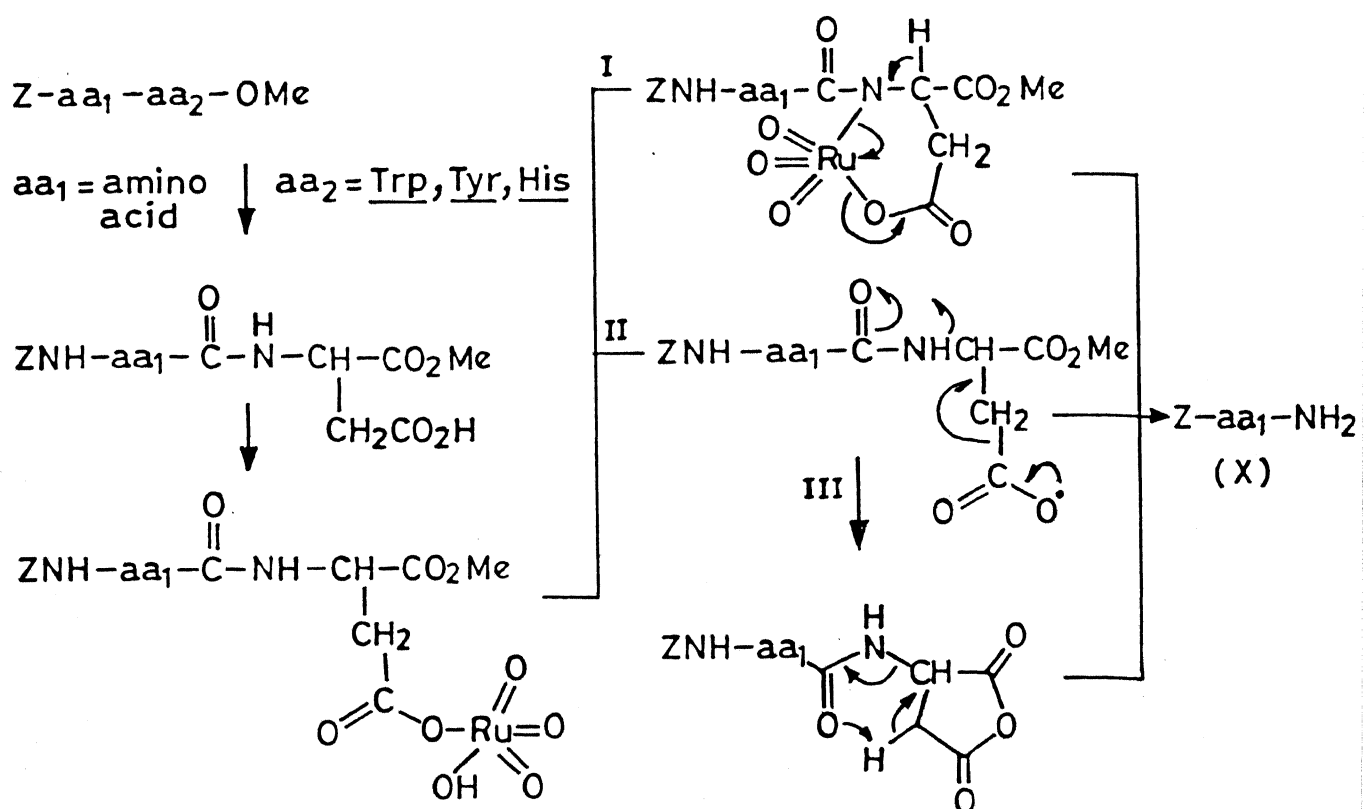
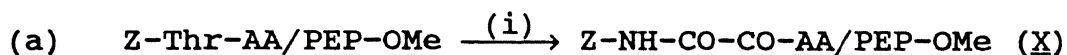
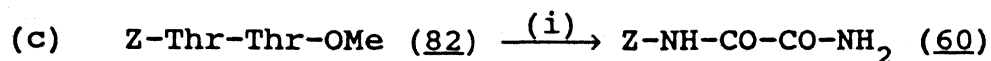
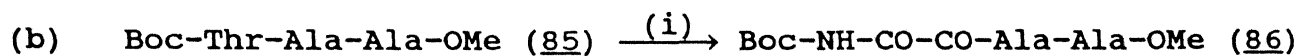


CHART C.12



AA = Amino Acid; PEP = Peptide

AA/PEP (No.)	(X)
Gly (<u>78</u>)	Z-NH-CO-CO-Gly-OMe (<u>50</u>)
Ala (<u>79</u>)	Z-NH-CO-CO-Ala-OMe (<u>52</u>)
Phe (<u>80</u>)	Z-NH-CO-CO-Phe-OMe (<u>54</u>)
Leu (<u>81</u>)	Z-NH-CO-CO-Leu-OMe (<u>56</u>)
Lys(N ^ω Z) (<u>83</u>)	Z-NH-CO-CO-Lys(N ^ω Z)-OMe (<u>84</u>)
Ala-Ala (<u>87</u>)	Z-NH-CO-CO-Ala-Ala-OMe (<u>88</u>)
Cys(S-Bzl) (<u>89</u>)	Z-NH-CO-CO-Cys(SO ₂ -Bzl)-OMe (<u>90</u>)
Leu-Leu (<u>91</u>)	Z-NH-CO-CO-Leu-Leu-OMe (<u>92</u>)
Leu-Leu-Leu (<u>93</u>)	Z-NH-CO-CO-Leu-Leu-Leu-OMe (<u>94</u>)



(i) NaIO₄/RuCl₃·3H₂O/MeCN:CCl₄:pH 3 buffer/1.5 h

The formation of Z-NH-CO-CO-Lys(N^ωZ)-OMe (84) in 60% yields is noteworthy. Hydrogenolysis here would readily afford NH₂-CO-CO-NH(COOMe)-(CH₂)₄-NH₂, a construct of appeal in protein design and cross linking. The excellent stability shown by the Boc protecting group to the conditions of reaction is seen in the transformation of Boc-Thr-Ala-Ala-OMe (85) to Boc-NH-CO-CO-Ala-Ala-OMe (86) in 90% yields. Here ester hydrolysis and coupling would provide a practical route to N-oxalamido peptides.

Z-NH-CO-CO-Lys(N^ωZ)-OMe (84): (60%)

mp. : syrup

ir : ν_{\max} (KBr)cm⁻¹: 3329 (NH), 1791 (CONHCO), 1700 (br, amide I), 1494 (br, amide II).

nmr : δ (CDCl₃): 1.03-1.93 (6H, m, Lys C^βH₂ + Lys C^γH₂ + Lys C^δH₂), 3.12 (2H, m, Lys C^ωH₂), 3.68 (3H, s, COOCH₃), 4.46 (1H, m, Lys C^αH), 5.09 (4H, s, Z CH₂x2), 7.34 (11H, brs + m, Lys N^ωH + aromatic protons), 7.90 (1H, d, J=7.5 Hz, exchangeable with D₂O, Lys N^αH), 9.43 (1H, brs, exchangeable, CONHCO).

anal: Found: C, 59.87; H, 5.82; N, 8.37 %

Calc. for C₂₅H₂₉N₃O₈: C, 60.12; H, 5.81; N, 8.42 %

Z-NH-CO-CO-Ala-Ala-OMe (88): (76%)

mp. : syrup

ir : ν_{\max} (KBr)cm⁻¹: 3328 (NH), 1793 (CONHCO), 1742 (ester), 1689 (amide I), 1487 (br, amide II).

nmr : δ (CDCl₃): 1.46 (6H, d, J=6.5 Hz, Ala CH₃x2), 3.78 (3H, s, COOCH₃), 4.56 (2H, m, Ala C^αHx2), 5.25 (2H, s, Z CH₂), 7.37 (6H, s, Ala NH + aromatic protons), 7.93 (1H, brd, Ala NH), 9.46 (1H, brs, CONHCO).

anal: Found: C, 53.47; H, 5.18; N, 11.33 %

Calc. for $C_{17}H_{21}N_3O_7$: C, 53.83; H, 5.54; N, 11.08 %

Z-NH-CO-CO-Cys(SO₂-Bzl)-OMe (90): (79%)

mp. : 101-102°C

ir : ν_{\max} (KBr)cm⁻¹: 3302 (NH), 1783 (CONHCO), 1743 (ester),
1673 (amide I), 1483 (br, amide II), 1311, 1173, 1134 (SO₂).

nmr : δ (CDCl₃): 3.40 (2H, d, J=5.0 Hz, Cys C ^{β} H₂), 3.78 (3H, s, COOCH₃), 4.28 (2H, s, Bzl CH₂), 4.96 (1H, m, Cys C ^{α} H), 5.25 (2H, s, Z CH₂), 7.43 (10H, s, aromatic protons), 8.40 (1H, d, J=7.5 Hz, exchangeable with D₂O, Cys NH), 9.37 (1H, s, exchangeable, CONHCO).

ms : m/z: 463 (MH)⁺.

anal: Found: C, 54.18; H, 4.68; N, 5.83 %

Calc. for $C_{21}H_{22}N_2O_8S$: C, 54.55; H, 4.76; N, 6.06 %

[α]_D²⁵: +1.82 (c, 1.7, CHCl₃).

Z-NH-CO-CO-Leu-Leu-OMe (92): (96%)

mp. : syrup

ir : ν_{\max} (neat)cm⁻¹: 3308 (NH), 1788 (CONHCO), 1744 (ester),
1665 (br, amide I), 1535 (amide II), 1484.

nmr : δ (CDCl₃): 0.93 (12H, brs, Leu CH₃×4), 1.62 (6H, m, Leu C ^{β} H₂×2 + Leu C ^{γ} H×2), 3.72 (3H, s, COOCH₃), 4.56 (2H, m, Leu C ^{α} H×2), 5.22 (2H, s, Z CH₂), 6.78 (1H, d, J=7.5 Hz, exchangeable with D₂O, Leu NH), 7.37 (5H, s, aromatic protons), 8.00 (1H, d, J=7.5 Hz, exchangeable, Leu NH), 9.62 (1H, s, exchangeable, CONHCO).

ms : m/z: 464 (MH)⁺.

anal: Found: C, 59.44; H, 7.23; N, 9.27 %

Calc. for $C_{23}H_{33}N_3O_7$: C, 59.61; H, 7.13; N, 9.07 %

Z-NH-CO-CO-Leu-Leu-Leu-OMe (94): (36%)

mp. : syrup

ir : ν_{\max} (neat) cm^{-1} : 3289 (NH), 1784 (CONHCO), 1742 (ester), 1651 (br, amide I), 1484 (br, amide II).

nmr : δ (300 MHz, CDCl_3): 0.9 (18H, brs, Leu $\text{CH}_3 \times 6$), 1.66 (9H, m, Leu $\text{C}^\beta \text{H}_2 \times 3$ + Leu $\text{C}^\gamma \text{H} \times 3$), 3.72 (3H, s, COOCH_3), 4.34-4.64 (3H, m, Leu $\text{C}^\alpha \text{H} \times 3$), 5.24 (2H, s, Z CH_2), 6.40 (2H, m, exchangeable with D_2O , Leu $\text{NH} \times 2$), 7.36 (5H, s, aromatic protons), 7.80 (1H, d, $J=7.5$ Hz, exchangeable, Leu NH), 9.40 (1H, s, exchangeable, CONHCO).

ms : m/z : 577 (MH)⁺.

anal: Found: C, 60.09; H, 7.73; N, 10.04 %

Calc. for $\text{C}_{29}\text{H}_{44}\text{N}_4\text{O}_8$: C, 60.42; H, 7.64; N, 9.72 %

Boc-NH-CO-CO-Ala-Ala-OMe (86): (92%)

mp. : syrup

ir : ν_{\max} (KBr) cm^{-1} : 3394 (NH), 3302 (NH), 1786 (CONHCO), 1746 (ester), 1660 (br, amide I), 1542 (amide II).

nmr : δ (CDCl_3): 1.00-1.62 (15H, m, Boc $\text{CH}_3 \times 3$ + Ala $\text{CH}_3 \times 2$), 3.79 (3H, s, COOCH_3), 4.54 (2H, m, Ala $\text{C}^\alpha \text{H} \times 2$), 6.73 (1H, d, $J=7.5$ Hz, Ala NH), 8.06 (1H, d, $J=7.5$ Hz, Ala NH), 9.28 (1H, s, CONHCO).

anal: Found: C, 48.53; H, 6.44; N, 12.27 %

Calc. for $\text{C}_{14}\text{H}_{23}\text{N}_3\text{O}_7$: C, 48.70; H, 6.67; N, 12.17 %

As in the case of the Ser, the Thr analog Z-Thr-Thr-OMe (82) failed to give products, arising from a combination of C-terminal amidation and N-terminal oxalamide formation. Here also the only compound isolated was Z-NH-CO-CO-NH₂ (60) arising from cleavage. In all likelihood, compound (60) arises from the rapid hydrolysis of expected Z-NH-CO-CO-NH-CO-NH₂ (Chart C.12.c).

Perhaps, the most dramatic outcome of the present study is the experimental demonstration of the transition state envisaged in Scheme

C.5.D.

The foregoing account has clearly shown the dichotomy in the Ru(VIII) mediated scission of Ser/Thr residues, namely, that carbinolamides having the profile of $-\text{CO}-\text{NH}-\text{CH}(\text{OH})-\text{COO}-$ undergoes cleavage and those of the type $-\text{CO}-\text{NH}-\text{CH}(\text{OH})-\text{CO}-\text{NH}-$ are oxidized to oxalamides, in preference to the cleavage. Logically then, the fate of the substrate is dictated by the C-terminal hetero atom; when this is 'O', amidation results and when the unit is 'NH', oxidation ensues. Fortunately, this aspect could be easily tested using C-terminal Ser/Thr amides in place of the C-terminal esters as the substrates. The expectations here based on transition state illustrated in Scheme C.5.D, namely, that C-terminal Ser/Thr amides would lead to oxalamides were indeed fully realized experimentally. Thus, a range of C-terminal Ser/Thr amides (Chart C.13) uniformly afforded in good to excellent yields the C-terminal amides. This aspect is highlighted below in Table C.1 comparing substrates that differ only at the C-terminal ends.

Table C.1

<u>Substrate</u>	<u>Product(Yield %)</u>	<u>Substrate</u>	<u>Product(Yield %)</u>
Bz-Ser-OMe	Bz-NH ₂ (74)	Bz-Ser-NH ₂	Bz-NH-CO-CO-NH ₂ (91)
Bz-Gly-Ser-OMe	Bz-Gly-NH ₂ (54)	Bz-Gly-Ser-NH ₂	Bz-Gly-NH-CO-CO-NH ₂ (90)
Bz-Leu-Ser-OMe	Bz-Leu-NH ₂ (68)	Z-Leu-Ser-NH ₂	Z-Leu-NH-CO-CO-NH ₂ (88)
Bz-Thr-OMe	Bz-NH ₂ (84)	Z-Thr-NH ₂	Z-NH-CO-CO-NH ₂ (61)

These endeavours reinforce the notion that conformational requirements pertaining to carbinolamide cleavage, namely, the anti-periplanar alignment of the OH and the CO units, is the key pertaining to the rationalization of the dichotomy. As illustrated in Scheme C.5.D, in the case of C-terminal amides, possibility for hydrogen bonding most likely, makes this requirement difficult with the result that the carbinolamides undergo preferential oxidation rather than cleavage.

As stated previously, many important biologically active peptides have C-terminal amide residues. Chart C.13 illustrates an excellent method for the otherwise difficultly preparable C-terminal oxalamides containing peptides, whose biological profile in selected cases would likely to be of great interest.

Bz-NH-CO-CO-NH₂ (96): (91%)

mp. : 111-112°C

ir : ν_{\max} (KBr)cm⁻¹: 3428 (NH), 3376 (NH), 3324 (NH), 3283 (NH), 3172 (NH), 1763 (CONHCO), 1702, 1678 (amide I), 1646 (amide I), 1597, 1578 (amide II), 1500.

nmr : δ (CDCl₃): 6.36 (2H, brd, exchangeable with D₂O, CONH₂), 7.15-8.12 (5H, m, aromatic protons), 10.56 (1H, s, exchangeable with D₂O, CONHCO).

ms : m/z: 193 (MH)⁺.

anal: Found: C, 56.08; H, 4.26; N, 14.96 %

Calc. for C₉H₈N₂O₃: C, 56.25; H, 4.17; N, 14.58 %

Z-Leu-NH-CO-CONH₂ (99): (88%)

mp. : 151-152°C

ir : ν_{\max} (KBr)cm⁻¹: 3396 (NH), 3304 (NH), 1764 (CONHCO), 1704, 1678 (amide I), 1518 (amide II).

nmr : δ [CDCl₃ + (CD₃)₂SO]: 0.93 (6H, d, J=5.0 Hz, Leu CH₃x2), 1.62 (3H, m, Leu C ^{β} H₂ + Leu C ^{γ} H), 4.65 (1H, m, Leu C ^{α} H), 5.18 (2H, s, Z CH₂), 6.71 (1H, d, J=7.5 Hz, exchangeable with D₂O, Leu NH), 7.27-7.75 (7H, s+br, CONH₂ + aromatic protons), 10.50 (1H, s, exchangeable, CONHCO).

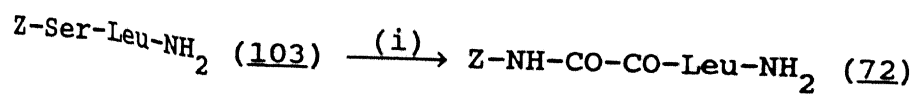
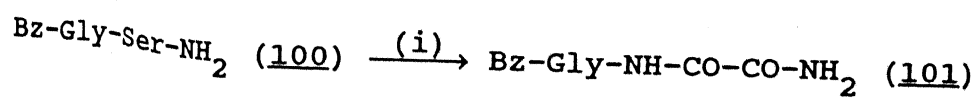
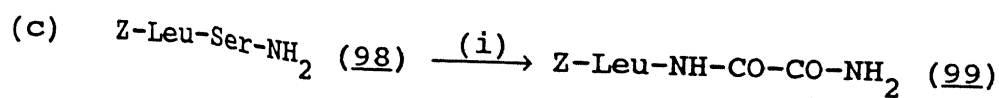
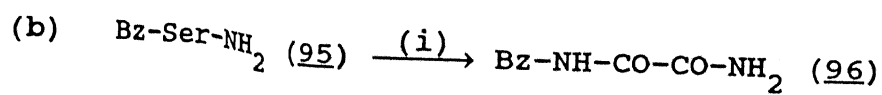
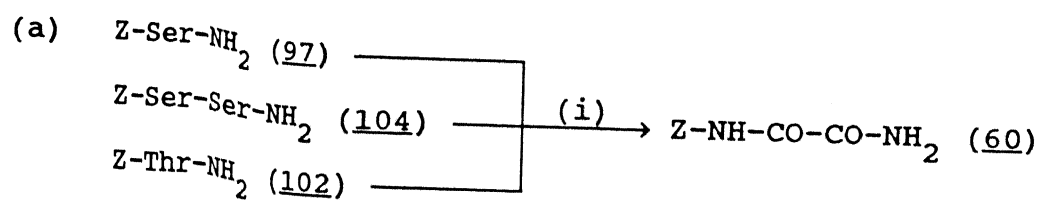
ms : m/z: 336 (MH)⁺.

anal: Found: C, 57.13; H, 6.48; N, 12.67 %

Calc. for C₁₆H₂₁N₃O₅: C, 57.31; H, 6.27; N, 12.54 %

[α]_D²⁶: -20.94 (c, 0.85, MeOH).

CHART C.13



Bz-Gly-NH-CO-CONH₂ (101): (90%)

mp. : 164-165°C

ir : ν_{\max} (KBr) cm⁻¹: 3397 (NH), 3288 (NH), 3160 (NH), 1780 (CONHCO), 1687 (amide I), 1637 (amide I), 1557 (amide II), 1490.

nmr : δ [CDCl₃ + (CD₃)₂SO]: 4.40 (2H, d, J=5.0 Hz, Gly CH₂), 6.71 (1H, br, exchangeable with D₂O, Gly NH), 7.00-8.46 (7H, m, CONH₂ + aromatic protons), 10.65 (1H, s, exchangeable, CONHCO).

ms : m/z: 250 (MH)⁺.

anal: Found: C, 52.81; H, 4.46; N, 17.16 %

Calc. for C₁₁H₁₁N₃O₄: C, 53.01; H, 4.42; N, 16.87 %

The most practical outcome arising from the understanding of factors that control the fate of the intermediate carbinolamide is the prediction that non-terminal Ser/Thr residues in peptides on treatment with Ru(VIII) would give rise to backbone modified peptides involving a -CO-NH-CH(CHR-OH)-CO-NH- \longrightarrow -CO-NH-CO-CO-NH- change. This expectation has been fully realized. In Chart C.14.a, the smooth transformation of the tripeptides of the profile P-AA-Ser-AA-OMe \longrightarrow P-AA-NH-CO-CO-AA-OMe are presented.

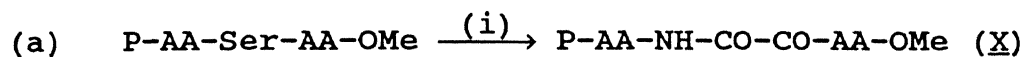
Bz-Leu-NH-CO-CO-Leu-OMe (106): (55%)

mp. : 75-77°C

ir : ν_{\max} (KBr) cm⁻¹: 3330 (NH), 1770 (CONHCO), 1745 (ester), 1690 (amide I), 1645 (amide I), 1605, 1578 (amide II), 1530 (amide II), 1470.

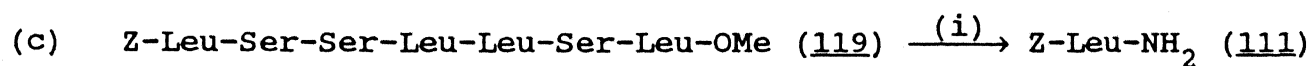
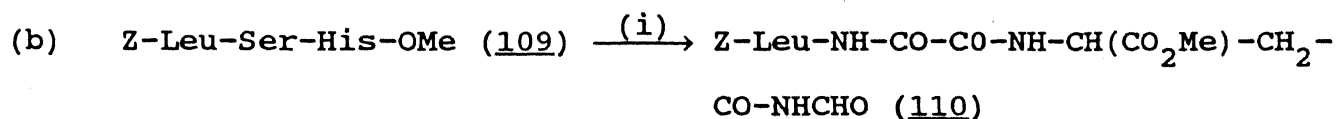
nmr : δ (CDCl₃): 0.93 (12H, d, J=5.0 Hz, Leu CH₃x4), 1.68 (6H, m, Leu C ^{β} H₂x2 + Leu C ^{γ} Hx2), 3.75 (3H, s, COOCH₃), 4.59 (1H, m, Leu C ^{α} H), 5.34 (1H, m, Leu C ^{α} H), 7.04 (1H, d, J=7.5 Hz, exchangeable with D₂O, Leu NH), 7.25-8.03 (6H, m, Leu NH + aromatic protons), 10.23 (1H, s, exchangeable, CONHCO).

CHART C.14



AA = Amino Acid

P	AA (No.)	(X)
Bz	Leu (<u>105</u>)	Bz-Leu-NH-CO-CO-Leu-OMe (<u>106</u>)
Bz	Ala (<u>107</u>)	Bz-Ala-NH-CO-CO-Ala-OMe (<u>108</u>)
Z	Gly (<u>112</u>)	Z-Gly-NH-CO-CO-Gly-OMe (<u>113</u>)
Bz	Pro (<u>114</u>)	Bz-Pro-NH-CO-CO-Pro-OMe (<u>115</u>)
Bz	Aib (<u>116</u>)	Bz-Aib-NH-CO-CO-Aib-OMe (<u>117</u>)



(i) $\text{NaIO}_4/\text{RuCl}_3 \cdot 3\text{H}_2\text{O}/\text{MeCN}:\text{CCl}_4:\text{pH } 3 \text{ buffer}/1.5 \text{ h}$

anal: Found: C, 61.28; H, 7.18; N, 9.36 %

Calc. for $C_{22}H_{31}N_3O_6$: C, 60.97; H, 7.16; N, 9.70 %

Bz-Ala-NH-CO-CO-Ala-OMe (108): (98%)

mp. : 140-141°C

ir : ν_{\max} (KBr) cm^{-1} : 3277 (NH), 1762 (CONHCO), 1740 (ester),
1674 (amide I), 1630 (amide I), 1534 (amide II), 1488.

nmr : δ (CDCl_3): 1.46, 1.51 (3H, 3H, d, d, $J=6.5$ Hz, 6.5 Hz, Ala
 $\text{CH}_3 \times 2$), 3.78 (3H, s, COOCH_3), 4.53 (1H, m, Ala C^αH), 5.31
(1H, m, Ala C^αH), 6.81 (1H, d, $J=7.5$ Hz, exchangeable with
 D_2O , Ala NH), 7.28-8.00 (6H, m, Ala NH + aromatic protons),
10.09 (1H, s, exchangeable, CONHCO).

ms : m/z : 349 (M)⁺.

anal: Found: C, 55.43; H, 5.08; N, 11.87 %

Calc. for $C_{16}H_{19}N_3O_6$: C, 55.01; H, 5.44; N, 12.03 %

$[\alpha]_D^{28}$: +1.2 (c, 1.6, CHCl_3).

Z-Gly-NH-CO-CO-Gly-OMe (113): (50%)

mp. : 115-116°C

ir : ν_{\max} (KBr) cm^{-1} : 3383 (NH), 3325 (NH), 3272 (NH), 3210 (NH),
3169 (NH), 1760 (CONHCO), 1720 (ester), 1690 (br,
carbamate), 1651 (amide I), 1531 (amide II), 1512 (amide
II), 1493.

nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 3.75 (3H, s, COOCH_3), 4.06, 4.34
(2H, 2H, d, d, $J=5.0$ Hz, 5.0 Hz, Gly $\text{CH}_2 \times 2$), 5.12 (2H, s, Z
 CH_2), 6.46 (1H, br, exchangeable with D_2O , Gly NH(Z)), 7.34
(5H, s, aromatic protons), 8.75 (1H, brm, exchangeable, Gly
NH), 10.25 (1H, s, exchangeable, CONHCO).

ms : m/z : 351 (M)⁺.

anal: Found: C, 50.87; H, 4.64; N, 11.79 %

Calc. for $C_{15}H_{17}N_3O_7$: C, 51.28; H, 4.84; N, 11.97 %

Bz-Pro-NH-CO-CO-Pro-OMe (115): (40%)

mp. : syrup

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3320 (br, NH), 1725 (ester), 1640 (amide I), 1600, 1560 (amide II).

nmr : δ (CDCl_3): 1.75-2.31 (8H, m, Pro $\text{C}^\beta\text{H}_2 \times 2$ + Pro $\text{C}^\gamma\text{H}_2 \times 2$), 3.59-3.93 (7H, s+m, COOCH_3 + Pro $\text{C}^\gamma\text{H}_2 \times 2$), 4.25-4.93 (2H, m, Pro $\text{C}^\alpha\text{H} \times 2$), 6.18 (1H, br, exchangeable with D_2O , CONHCO), 7.28-8.00 (5H, m, aromatic protons).

$[\alpha]_{\text{D}}^{28}$: -38.18 (c, 0.27, CHCl_3).

Bz-Aib-NH-CO-CO-Aib-OMe (117): (86%)

mp. : 173-174°C

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3377 (NH), 3326 (NH), 1770 (CONHCO), 1738 (ester), 1702, 1680 (amide I), 1657 (amide I), 1519 (amide II).

nmr : δ (CDCl_3): 1.66 (12H, s, s, Aib $\text{CH}_3 \times 2$; $\text{CH}_3 \times 2$), 3.71 (3H, s, COOCH_3), 6.78 (1H, s, Aib NH(Bz)), 7.25-8.00 (6H, m, Aib NH + aromatic protons), 10.68 (1H, s, CONHCO).

ms : m/z: 378 (MH)⁺.

anal: Found: C, 57.46; H, 6.27; N, 11.26 %

Calc. for $\text{C}_{18}\text{H}_{23}\text{N}_3\text{O}_6$: C, 57.29; H, 6.10; N, 11.14 %

The reaction here affords exclusively the expected oxalamides and provides a practical route to peptide backbone modification. This is particularly evident in the case of the Bz-Pro-Ser-Pro-OMe (114) \rightarrow Bz-Pro-NH-CO-CO-Pro-OMe (115) change (Chart C.14.a).

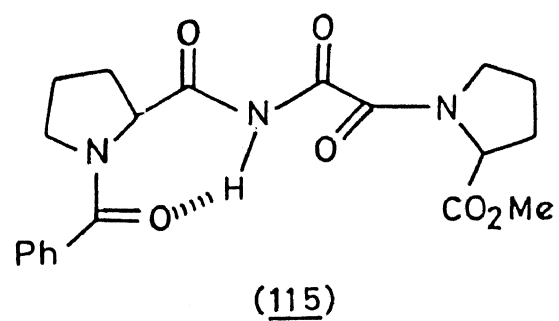
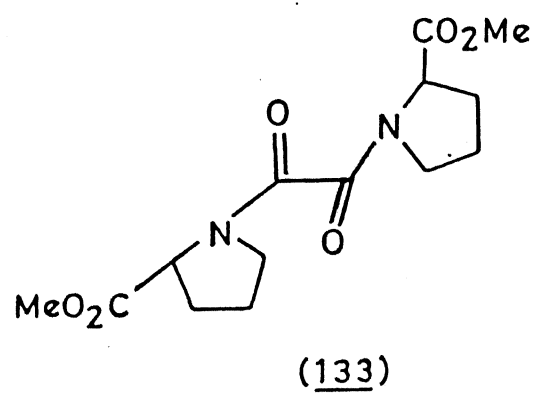
It may be noted that the peptide backbone modification arising from oxidation of non-terminal Ser residues leads to incorporation of the -CO-NH-CO-CO-NH-(CH(R))- unit, the core of which is the retropeptidomimetic -NH-CO-CO-NH- (oxalamido) moiety. Here, the modulation of the -CO-CO- dihedral angle from one having a perfect C_2 symmetry to an

orthogonal alignment has implications pertaining to transition state associated with rotamase activity and immune suppression. In this connection, in the present work, a range of peptides containing the core retropeptido oxalamide unit was prepared (*vide infra*). Pertinent to the present discussion is the preparation and the X-ray crystallographic study of MeO-Pro-CO-CO-Pro-OMe (133). Whilst in normal retropeptido-mimetic oxalamides (Aib, Leu) the CO-CO dihedral angle was close to 180° (C_2 symmetry), in the case of (133), it was 106° , close to orthogonality. A comparative spectral analysis of compounds listed in Chart C.14.a, tend to show that the conformation of Bz-Pro-NH-CO-CO-NH-Pro-OMe (115) is quite different from that of others. Most profound is the upfield shift exhibited by the -CO-NH-CO-CO-CH(R)- proton from approximately 10.2 ppm to 6 ppm! Additionally, in the IR, the 1770 cm^{-1} band found in the other cases of this class was absent in (115). It is very likely that compound (115) has a conformation similar to that of (133) as shown in Scheme C.8, with predictable three dimensional architecture that could play an important role in de-novo protein design.

Interestingly, Z-Leu-Ser-His-OMe (109) when subjected to Ru(VIII) treatment afforded Z-Leu-NH-CO-CO-NH-CH(CO₂Me)-CH₂-CO-NH-CHO (110), arising from the expected change of the serine residue and the oxidation of the histidine unit. This change is appealing in the sense that the same reaction brings about both peptide backbone modification and side chain transformation. Work from this laboratory¹⁷⁸ has shown that Z-His-OMe is transformed largely to the novel Z-NH-CH(CO₂Me)-CH₂-CO-NH-CHO via the sequence 4-5 π bond oxidative scission of the His imidazole ring, water addition and oxidation. The present illustration shows that such transformations do take place in a peptide environment also (Chart C.14.b).

Z-Leu-NH-CO-CO-NH-CH(CO₂Me)-CH₂-CO-NH-CHO (110): (73%)

pale yellow crystals from EtOAc/hexane.

Scheme C.8

mp. : 79-85°C

ir : ν_{\max} (KBr) cm^{-1} : 3396 (NH), 3319 (NH), 3209 (NH), 1775 (CONHCO), 1732 (ester), 1660 (amide I), 1543 (amide II).

nmr : δ (CDCl_3): 0.90 (6H, br, Leu $\text{CH}_3 \times 2$), 1.57 (3H, m, Leu $\text{C}^\beta \text{H}_2$ + Leu $\text{C}^\gamma \text{H}$), 3.14 (2H, m, $\text{C}^\beta \text{H}_2$ of N-formyl asparagine), 3.78 (3H, s, COOCH_3), 4.87 (2H, m, Leu $\text{C}^\alpha \text{H}$ + $\text{C}^\alpha \text{H}$ of N-formyl asparagine), 5.10 (2H, s, Z CH_2), 5.50 (1H, br, exchangeable with D_2O , Leu NH), 7.34 (5H, s, aromatic protons), 8.40 (1H, d, $J=7.5$ Hz, exchangeable with D_2O , N-formyl asparagine $\text{N}^\alpha \text{H}$), 9.12 (1H, d, non-exchangeable, CHO), 10.18 (1H, s, exchangeable, CONHCO), 10.43 (1H, d, $J=7.5$ Hz, exchangeable, NHCHO).

The amphipathic heptapeptide Z-Leu-Ser-Ser-Leu-Leu-Ser-Leu-OMe (119), a heptamer repeat of an ion channel forming 21 residue peptide, was constructed as a model for extensive peptide backbone modification. Unfortunately, oxidation of this afforded only Z-Leu- NH_2 (111) (Chart C.14.c). Such problems have also been previously noted when contiguous Ser-Ser or Thr-Thr units are present in the chain (*vide supra*). The complication arises from the fact that the Ser-Ser or Thr-Thr residues here could be transformed to $-\text{NH}-\text{CO}-\text{CO}-\text{NH}-\text{CO}-\text{CO}-$, forming an extended stretch of oxalamide units. All indications are that such an ensemble is susceptible to hydrolytic cleavage resulting in the formation of a terminal amide. Viewed from a positive vantage, the present method can be used with confidence for peptide scission at Ser-Ser or Thr-Thr sites.

Z-Leu- NH_2 (111):

mp. : 117-118°C (lit.¹⁶⁶ mp. 125-126°C)

ir : ν_{\max} (KBr) cm^{-1} : 3391 (NH), 3321 (NH), 3191 (NH), 1657 (amide I), 1543 (amide II).

nmr : δ (CDCl_3): 0.90 (6H, d, $J=5.0$ Hz, Leu $\text{CH}_3 \times 2$), 1.62 (3H, m,

Leu C^βH₂ + Leu C^γH), 4.18 (1H, m, Leu C^αH), 5.09 (2H, s, 2 CH₂), 5.31 (1H, d, J=7.5 Hz, exchangeable with D₂O, Leu NH), 5.90 (2H, br, exchangeable, CONH₂), 7.34 (5H, s, aromatic protons).

anal: Found: C, 63.43; H, 7.29; N, 10.43 %

Calc. for C₁₄H₂₀N₂O₃: C, 63.64; H, 7.58; N, 10.61 %

Parenthetically, although oxalamides are more susceptible to cleavage to amides than normal peptides, control experiments with the model R-NH-CO-CO₂Me have clearly established that the terminal peptides, reported in the present work and produced in good yields, do not go through oxalamide intermediates.

Chart C.15.a and Chart C.15.b illustrate the smooth transformation of non-terminal Thr residues to backbone modified peptides. It is interesting to note that in the NMR of (123), the -CO-NH-CO-CO- proton is shifted upfield to 8 ppm from the normal 10.5 ppm indicating the likely distortion of the CO-CO dihedral angle from 180° (*vide supra*).

Bz-Ala-Ala-NH-CO-CO-Ala-Ala-OMe (123): (20%)

mp. : gummy

ir : ν_{\max} (KBr) cm⁻¹: 3295 (br, NH), 1756 (br, CONHCO, ester), 1637 (amide I), 1548 (amide II).

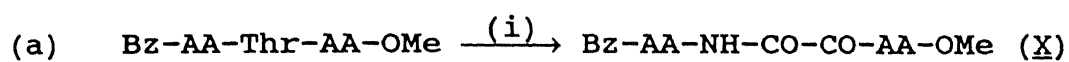
nmr : δ (CDCl₃): 1.40 (12H, d, J=6.5 Hz, Ala CH₃x4), 3.78 (3H, s, COOCH₃), 4.59 (4H, m, Ala C^αHx4), 6.43-8.28 (10H, m, Ala NHx4 + CONHCO + aromatic protons).

ms : m/z: 492 (MH)⁺.

$[\alpha]_D^{28}$: -18.97 (c, 1.66, MeOH).

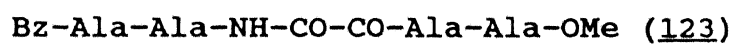
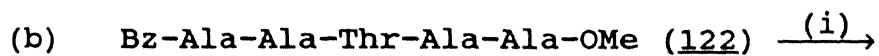
The rationalization of dichotomy in α -amidation versus oxalamide formation, on the basis of transition state envisaged in Scheme C.5, has found extensive experimental support. Scheme C.9 summarizes the finding pertaining to the novel C^α-C Ser/Thr side chain scission. The

CHART C.15



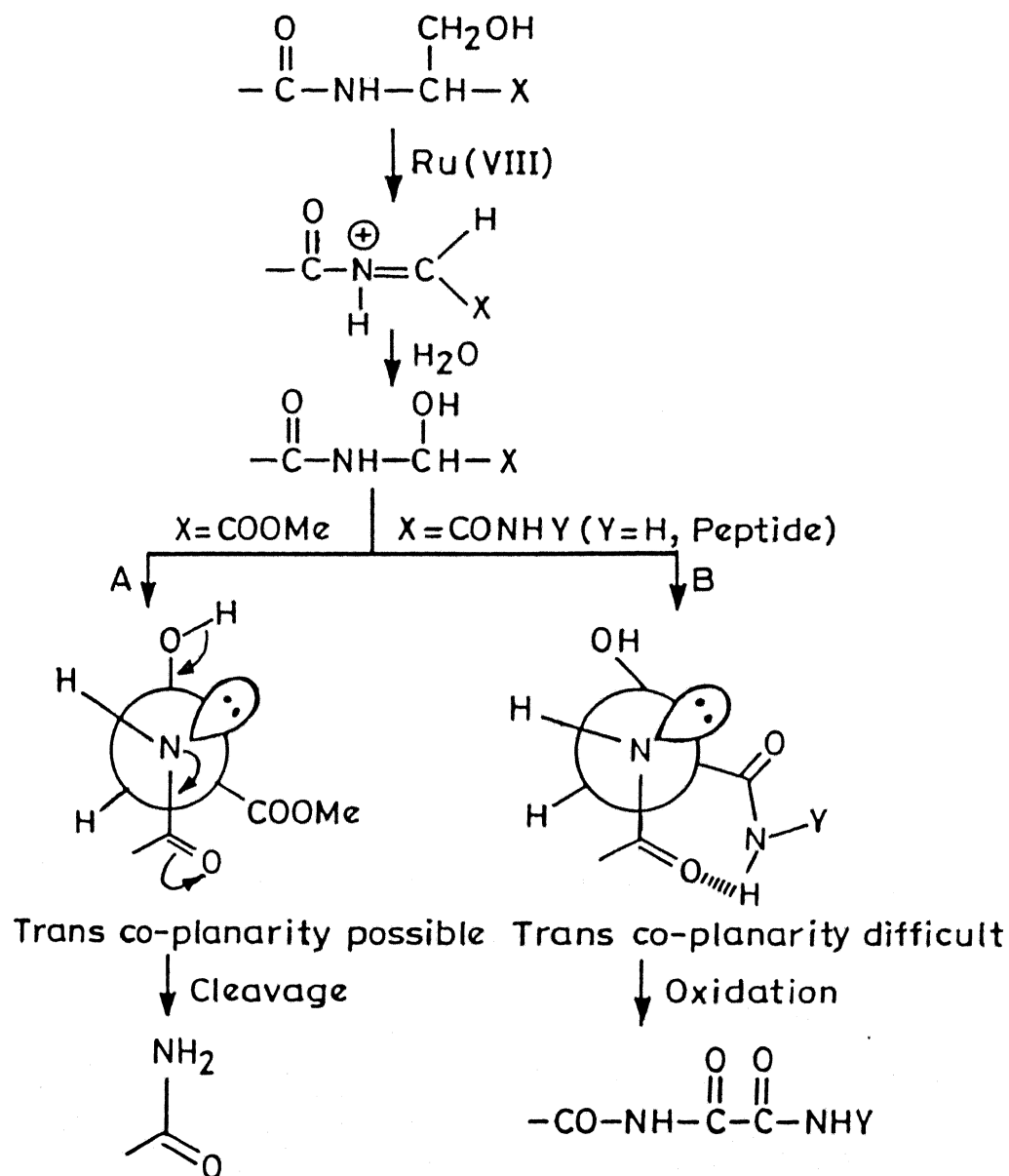
AA = Amino Acid

AA (No.)	(<u>X</u>)
Ala (<u>120</u>)	Bz-Ala-NH-CO-CO-Ala-OMe (<u>108</u>)
Pro (<u>121</u>)	Bz-Pro-NH-CO-CO-Pro-OMe (<u>115</u>)



(i) NaIO₄/RuCl₃·3H₂O/MeCN:CCl₄:pH 3 buffer/1.5 h

Scheme C.9 Rationalization of Dichotomy in α -amidation vs oxalamide formation



comprehensive study, outlined above, should add a new dimension pertaining to peptide/protein modification, an area of current interest, arising from the potential application of such altered substrates in diverse domains such as protein design, protein conformation, protein engineering and enzyme inhibitors. An aspect that has not found appreciation is that in large peptides the usual methods for C-terminal amidation gives poor results and it is quite possible that PAM mediated terminal amidation from Gly extended precursors evolved precisely for this reason. In this context our chemical simulation studies would have practical implications relating to the preparation of terminal amides of larger peptides.

A mechanistically pleasing aspect of this work is the fate of carbinolamide intermediate which is delicately poised either toward cleavage or further oxidation. The present work has endeavoured to rationalize this dichotomy and in this process has evolved a very reliable strategy for peptide backbone modification.

The serendipitous finding, namely that, $-\text{CH}_2\text{COOH}$ unit arising from oxidation of Trp/Tyr residues can also bring about terminal amidation possibly via carbinolamides is significant in that, this not only provides methodologies for protein chain rupture at these sites but also identifies a new class of coded amino acids as Gly equivalents in PAM reaction.

In spite of continuing interest in the chemistry of peptides and proteins, endeavours leading to significant alteration of the peptide backbone has not received due attention (see, Section B). The methodology delineated here leading to the placement of oxalamide units in intact peptide chains is bound to have ramifications across the peptide protein domain.

A significant outcome of endeavours described above is the generation of, in the peptide backbone, the unit $-\text{CO}-\text{NH}-\text{CO}-\text{CO}-\text{NH}-\text{CH}(\text{R})-$. The realization came early to the effect that the incorporation of such a unit

transforms a normal peptide into a retropeptide and that the core element here is the retropeptido-mimetic oxalamido unit, NH-CO-CO-NH . Apart from implications pertaining to backbone modifications, it was recognized that the $-\text{NH-CO-CO}-$ unit here is in an integral part of immune suppressors such as rapamycin, FK-506 and related antibiotics and that the conformation around the CO-CO unit plays a pivotal role in the sense that when the normal 180° dihedral angle here is restrained to approximately 90° , because of its resemblance to the twisted transition state, prolyl $\text{cis} \rightleftharpoons \text{trans}$ isomerism (rotamase activity) is inhibited.

Thus, it was envisaged that the preparation of a range of peptides having the retropeptido oxalamido unit and the study of their conformation would be of relevance not only with respect to endeavours described above but also in the development of rotamase inhibitor mimics.

Interestingly, the direct strategy to prepare such compounds by simple condensation of N-terminal free peptides with oxalyl chloride failed. Consequently, an alternate strategy consisting of two parts, namely, the construction of the core element and further elongation of the central motif had to be evolved. The core element, represented by $(\text{CO-A}_{\text{aa}}-\text{OMe})_2$ (aa = amino acid A), was constructed by the reaction of *in situ* generated appropriate α -amino acid ester with oxalyl chloride in excellent yields. A range of core oxalamido peptides, thus prepared, is shown in Chart C.16. Of particular interest here is the ready formation of such compounds involving methionine, tyrosine, tryptophan, proline and N^α -protected lysine (Chart C.16.a, C.16.c and C.16.d).

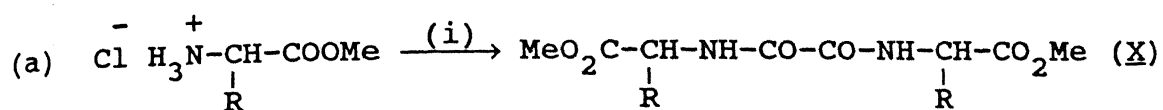
$\text{MeO-Gly-CO-CO-Gly-OMe}$ (124): (86%)

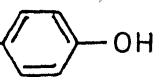
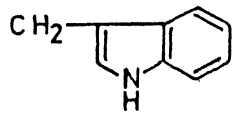
mp. : crystals from methanol, $120-121^\circ\text{C}$ (lit.¹⁷¹ mp. $159-160^\circ\text{C}$)

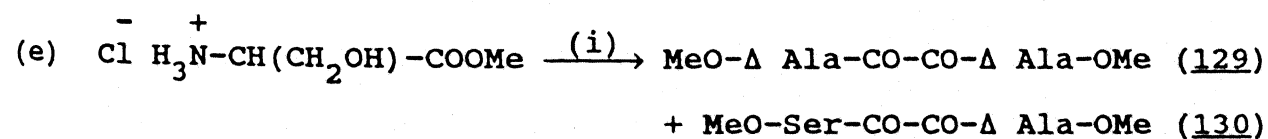
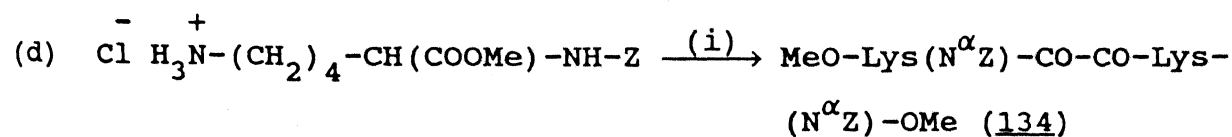
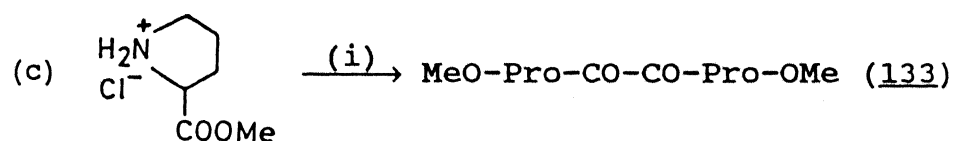
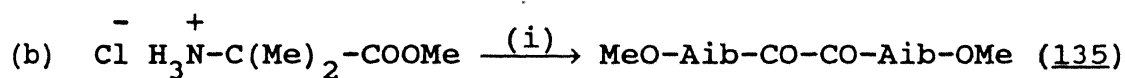
ir : ν_{max} (KBr) cm^{-1} : 3373 (NH), 1737 (ester), 1680 (amide I), 1507 (amide II), 1443, 1406, 1372.

nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 3.73 (6H, s, $\text{COOCH}_3 \times 2$), 4.00 (4H, d, $\text{J}=6.0$ Hz, Gly $\text{CH}_2 \times 2$), 8.82 (2H, t, exchangeable with D_2O ,

CHART C.16



R	(X)
H	MeO-Gly-CO-CO-Gly-OMe (<u>124</u>)
CH ₃	MeO-Ala-CO-CO-Ala-OMe (<u>125</u>)
CH ₂ Ph	MeO-Phe-CO-CO-Phe-OMe (<u>126</u>)
CH ₂ CH(Me) ₂	MeO-Leu-CO-CO-Leu-OMe (<u>127</u>)
CH ₂ CH ₂ SMe	MeO-Met-CO-CO-Met-OMe (<u>128</u>)
CH ₂ -  -OH	MeO-Tyr-CO-CO-Tyr-OMe (<u>131</u>)
CH ₂ - 	MeO-Trp-CO-CO-Trp-OMe (<u>132</u>)



(i) (COCl)₂/NEt₃/CH₂Cl₂/0°; 0.5 h/rt/12 h

Gly NHx2).

ms : m/z: 233 (MH)⁺, 117 (M/2 + H)⁺.

anal: Found: C, 41.46; H, 5.18; N, 12.33 %

Calc. for C₈H₁₂N₂O₆: C, 41.38; H, 5.17; N, 12.07 %

MeO-Ala-CO-CO-Ala-OMe (125): (55%)

mp. : crystals form EtOAc/hexane, 156-60°C (lit.¹⁷³ mp. 166-167°C)

ir : ν_{\max} (KBr)cm⁻¹: 3279 (NH), 1737 (ester), 1659 (amide I),
1531 (amide II), 1449.

nmr : δ (60 MHz, CDCl₃): 1.50 (6H, d, J=6.5 Hz, Ala CH₃x2), 3.76
(6H, s, COOCH₃x2), 4.50 (2H, m, Ala C ^{α} Hx2), 7.76 (2H, br,
exchangeable with D₂O, Ala NHx2).

ms : m/z: 261 (MH)⁺.

anal: Found: C, 45.86; H, 6.48; N, 11.77 %

Calc. for C₁₀H₁₆N₂O₆: C, 46.15; H, 6.15; N, 10.77 %

[α]_D²⁴: -73.45 (c, 0.55, MeOH).

MeO-Phe-CO-CO-Phe-OMe (126): (60%)

mp. : 192-193°C

ir : ν_{\max} (KBr)cm⁻¹: 3284 (NH), 1738 (ester), 1661 (amide I),
1523 (amide II), 1440.

nmr : δ (CDCl₃): 3.15 (4H, d, J=6.25 Hz, Phe C ^{β} H₂x2), 3.73 (6H,
s, COOCH₃x2), 4.80 (2H, m, Phe C ^{α} Hx2), 7.31 (10H, s,
aromatic protons), 7.75 (2H, d, J=7.5 Hz, exchangeable,
Phe NHx2).

ms : m/z: 413 (MH)⁺, 206 (M/2)⁺.

anal: Found: C, 64.17; H, 5.71; N, 6.96 %

Calc. for C₂₂H₂₄N₂O₆: C, 64.08; H, 5.83; N, 6.80 %

[α]_D²⁴: +28.00 (c, 0.10, MeOH).

MeO-Leu-CO-CO-Leu-OMe (127): (88%)

mp. : crystals from methanol, 114-115°C

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3345 (NH), 3299 (NH), 1741 (ester), 1664 (amide I), 1516 (amide II), 1437.

nmr : δ (CDCl_3): 0.93 (12H, d, $J=5.0$ Hz, Leu $\text{CH}_3 \times 4$), 1.64 (6H, m, Leu $\text{C}^\beta\text{H}_2 \times 2$ + Leu $\text{C}^\gamma\text{H} \times 2$), 3.73 (6H, s, $\text{COOCH}_3 \times 2$), 4.57 (2H, m, Leu $\text{C}^\alpha\text{H} \times 2$), 7.73 (2H, d, $J=7.5$ Hz, exchangeable, Leu $\text{NH} \times 2$).

ms : m/z : 345 (MH)⁺, 172 ($\text{M}/2$)⁺.

anal: Found: C, 56.14; H, 8.22; N, 8.48 %

Calc. for $\text{C}_{16}\text{H}_{28}\text{N}_2\text{O}_6$: C, 55.81; H, 8.14; N, 8.14 %

$[\alpha]_{\text{D}}^{24}$: -55.18 (c, 1.66, MeOH).

MeO-Met-CO-CO-Met-OMe (128): (45%)

mp. : crystals from methanol, 94-96°C

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3288 (NH), 1743 (ester), 1659 (amide I), 1524 (amide II), 1437.

nmr : δ (CDCl_3): 2.12 (10H, s+m, Met $\text{C}^\beta\text{H}_2 \times 2$ + Met $\text{S-CH}_3 \times 2$), 2.50 (4H, m, Met $\text{C}^\gamma\text{H}_2 \times 2$), 3.78 (6H, s, $\text{COOCH}_3 \times 2$), 4.68 (2H, m, Met $\text{C}^\alpha\text{H} \times 2$), 7.93 (2H, d, $J=8.00$ Hz, Met $\text{NH} \times 2$).

ms : m/z : 381 (MH)⁺, 190 ($\text{M}/2$)⁺.

anal: Found: C, 44.43; H, 6.17; N, 7.43 %

Calc. for $\text{C}_{14}\text{H}_{24}\text{N}_2\text{O}_6\text{S}_2$: C, 44.21; H, 6.32; N, 7.37 %

$[\alpha]_{\text{D}}^{24}$: -45.00 (c, 0.50, MeOH).

MeO-Tyr-CO-CO-Tyr-OMe (131): (88%)

mp. : crystals from methanol, 232-233°C

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3283 (NH), 1738 (ester), 1660 (amide I), 1520 (amide II), 1440.

nmr : δ [60 MHz, CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 3.03 (4H, d, $J=6.0$ Hz, Tyr $\text{C}^\beta\text{H}_2 \times 2$), 3.70 (6H, s, $\text{COOCH}_3 \times 2$), 4.70 (2H, m, Tyr $\text{C}^\alpha\text{H} \times 2$), 6.80 (8H, m, aromatic protons), 7.86 (2H, d, $J=7.5$ Hz, exchangeable, Tyr $\text{NH} \times 2$).

ms : m/z: 445 (MH)⁺.

anal: Found: C, 59.63; H, 5.38; N, 6.64 %

Calc. for C₂₂H₂₄N₂O₈: C, 59.46; H, 5.41; N, 6.31 %

[α]_D²⁴: +24.61 (c, 0.52, MeOH).

MeO-Trp-CO-CO-Trp-OMe (132): (60%)

mp. : white flakes from methanol, 177-178°C

ir : ν_{max} (KBr)cm⁻¹: 3410 (NH), 3298 (NH), 1740 (ester), 1661 (amide I), 1518 (amide II), 1457, 1438.

nmr : δ [CDCl₃ + (CD₃)₂SO]: 3.34 (4H, d, J=5.0 Hz, Trp C^βH₂x2), 3.71 (6H, s, COOCH₃x2), 4.78 (2H, m, Trp C^αHx2), 6.84-7.65 (10H, m, aromatic protons), 8.15 (2H, d, J=7.5 Hz, exchangeable, Trp NHx2), 10.34 (2H, s, exchangeable, Indole NHx2).

ms : m/z: 491 (MH)⁺, 245 (M/2)⁺.

anal: Found: C, 63.73; H, 5.21; N, 11.45 %

Calc. for C₂₆H₂₆N₄O₆: C, 63.67; H, 5.31; N, 11.43 %

[α]_D²⁴: +8.9 (c, 0.55, MeOH).

MeO-Aib-CO-CO-Aib-OMe (135): (70%)

mp. : crystals from methanol, 167-168°C

ir : ν_{max} (KBr)cm⁻¹: 3297 (NH), 1729 (ester), 1671 (amide I), 1504 (amide II).

nmr : δ [400 MHz, (CD₃)₂SO]: 1.41 (12H, s, Aib CH₃x4), 3.62 (6H, s, COOCH₃x2), 8.90 (2H, s, exchangeable, Aib NHx2).

ms : m/z: 289 (MH)⁺.

anal: Found: C, 50.29; H, 6.83; N, 10.18 %

Calc. for C₁₂H₂₀N₂O₆: C, 50.00; H, 6.94; N, 9.72 %

MeO-Pro-CO-CO-Pro-OMe (133): (70%)

mp. : crystals from ethyl acetate, 148-9°C (lit.¹⁷² mp. 154-155°C)

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 1740 (ester), 1662 (amide I), 1639 (amide I), 1541 (amide II).

nmr : δ (CDCl_3): 2.07 (8H, m, Pro $\text{C}^\beta\text{H}_2 \times 2$ + Pro $\text{C}^\gamma\text{H}_2 \times 2$), 3.81 (10H, s + m, Pro $\text{C}^\delta\text{H}_2 \times 2$ + $\text{COOCH}_3 \times 2$), 4.53, 5.87 (2H, m, m, Pro $\text{C}^\alpha\text{H} \times 2$).

ms : m/z: 313 (MH)⁺.

anal: Found: C, 53.83; H, 6.94; N, 8.62 %

Calc. for $\text{C}_{14}\text{H}_{20}\text{N}_2\text{O}_6$: C, 53.85; H, 6.41; N, 8.97 %

$[\alpha]_D^{24}$: -81.68 (c, 1.66, MeOH).

MeO-Lys(N^αZ)-CO-CO-Lys(N^αZ)-OMe (134): (44%)

mp. : 124-125°C

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3290 (NH), 1735 (ester), 1652 (amide I), 1512 (amide II).

nmr : δ (60 MHz, CDCl_3): 1.46 (12H, m, Lys $\text{C}^\beta\text{H}_2 \times 2$ + Lys $\text{C}^\gamma\text{H}_2 \times 2$ + Lys $\text{C}^\delta\text{H}_2 \times 2$), 3.23 (4H, m, Lys $\text{C}^\omega\text{H}_2 \times 2$), 3.70 (6H, s, $\text{COOCH}_3 \times 2$), 4.36 (2H, m, Lys $\text{C}^\alpha\text{H} \times 2$), 5.03 (4H, s, Z $\text{CH}_2 \times 2$), 5.53 (2H, d, J=7.5 Hz, exchangeable, Lys $\text{N}^\omega\text{H} \times 2$), 7.23 (10H, s, aromatic protons), 7.60 (2H, m, exchangeable, $\text{N}^\alpha\text{H} \times 2$).

ms : m/z: 643 (MH)⁺.

anal: Found: C, 59.49; H, 6.28; N, 8.29 %

Calc. for $\text{C}_{32}\text{H}_{42}\text{N}_4\text{O}_{10}$: C, 59.81; H, 6.54; N, 8.72 %

Interesting results were obtained when Ser-OMe was used as substrate. Here, in addition to the oxalamide formation the serine side chain was transformed into a dehydro alanine unit, giving rise to MeO- Δ Ala-CO-CO- Δ Ala-OMe (129) and MeO-Ser-CO-CO- Δ Ala-OMe (130) (Chart C.16.e). It was

$\text{MeO}_2\text{C}-\text{C}(=\text{CH}_2)-\text{NH}-\text{CO}-\text{CO}-\text{NH}-\text{C}(=\text{CH}_2)-\text{CO}_2\text{Me}$, (129): (14%)

mp. : 133-134°C

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3355 (NH), 3320 (NH), 1712 (ester), 1678 (amide I), 1620 (amide I), 1590, 1485 (br, amide II), 1430.

nmr : δ (CDCl₃): 3.84 (6H, s, COOCH₃x2), 6.00, 6.62 (2H, 2H, s, s, (=CH₂)x2), 9.56 (2H, s, exchangeable, NHx2).

ms : m/z: 257 (MH)⁺, 128 (M/2)⁺.

anal: Found: C, 47.16; H, 4.48; N, 10.83 %

Calc. for C₁₀H₁₂N₂O₆: C, 46.87; H, 4.69; N, 10.94 %

MeO₂C-CH(CH₂-OH)-NH-CO-CO-NH-C(=CH₂)-CO₂Me (130): (10%)

mp. : syrup

ir : ν_{\max} (KBr)cm⁻¹: 3360 (br, NH), 1725 (ester), 1660 (amide I), 1500 (amide II), 1432.

nmr : δ (CDCl₃): 3.84, 3.90 (3H, 3H, s, s, COOCH₃x2), 4.00 (2H, m, Ser C ^{β} H₂), 4.65 (1H, m, Ser C ^{α} H), 6.09, 6.68 (1H, 1H, s, s, =CH₂), 8.28 (1H, d, J=7.5 Hz, exchangeable, Ser NH), 9.56 (1H, s, exchangeable, Δ Ala NH).

ms : m/z: 275 (MH)⁺.

anal: Found: C, 44.04; H, 5.26; N, 9.93 %

Calc. for C₁₀H₁₄N₂O₇: C, 43.80; H, 5.11; N, 10.22 %

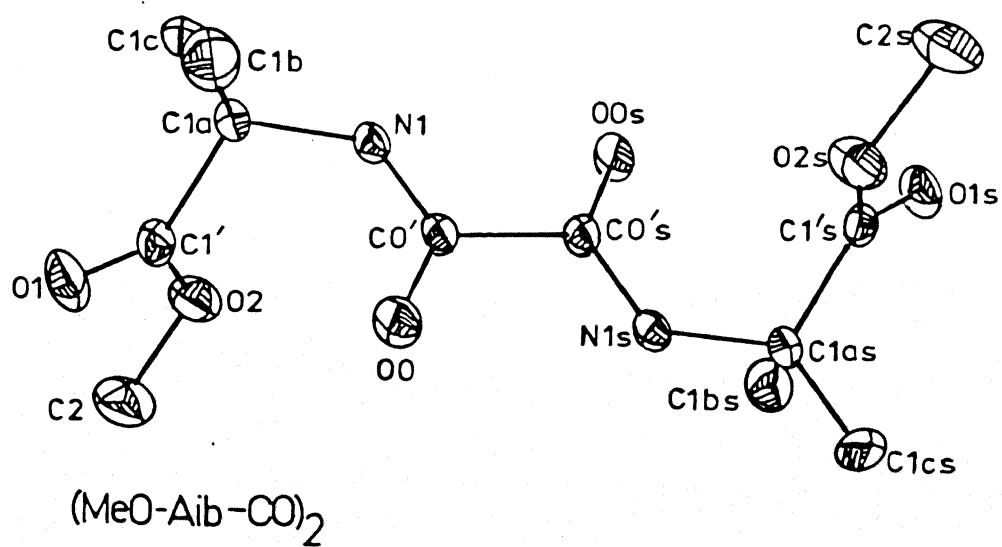
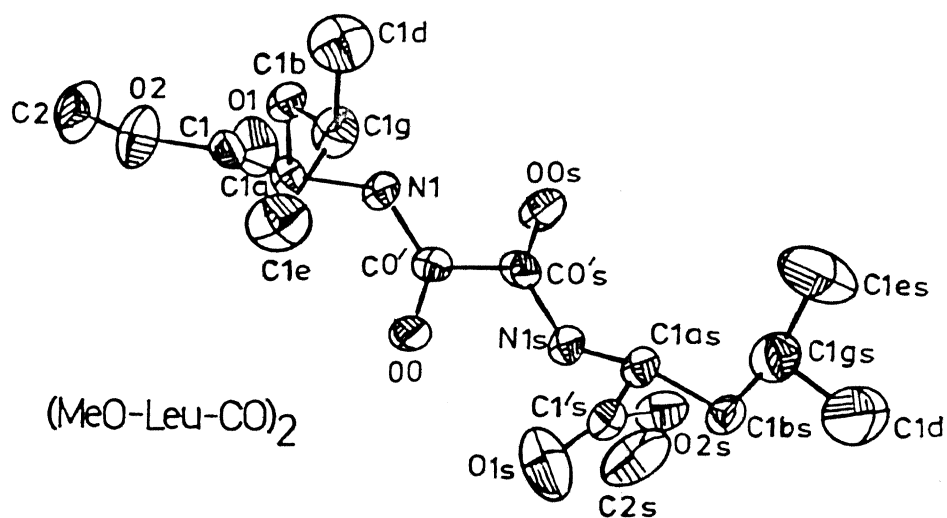
soon realized that this change constituted a novel observation in the sense that it afforded the ready preparation of Δ Ala units in a peptide environment from serine precursors and particularly so in view of the fact that the existing alternate procedures are very cumbersome. Endeavours to experimentally illustrate this, has provided very successful results which are reported at a later portion of the thesis. The transformation of Ser-OMe to (129) and (130) also provide a good method for core motifs containing Δ Ala residues.

The delineation of precise structures of unrestrained oxalamides was considered important since the retro-bispeptide unit would normally be expected to be planar (from a molecular orbital picture, the energetics would dictate the oxalamide system to be a single unit). However, this would not be possible in the event the two peptide units become

orthogonal, an arrangement shown for α -keto amide unit when present as part of a cyclic system such as those in the immunosuppressants, rapamycin and FK-506. It was therefore considered important that the information pertaining to the structure of these units would be of significance in the design of enzyme mimics and potential inhibitors. With this background in view, the X-ray crystal structures of three core oxaloptides namely MeO-Leu-CO-CO-Leu-OMe (127), MeO-Aib-CO-CO-Aib-OMe (135) and MeO-Pro-CO-CO-Pro-OMe (133) (Chart C.16), which form a nice set suitable for appropriate comparison, in the sense that it would bring about consequences of perturbation in the oxalamide environment arising from increasingly sterically encumbered side chains, was determined.

Colourless crystals were grown by slow evaporation from MeOH-H₂O solutions. Conformations found in the crystalline state for (127), (135) and (133) are shown in Scheme C.10. Scheme C.10 clearly shows that the oxalamide group has the trans conformation in two retropeptides and an approximately orthogonal conformation in the peptide with Pro residues. Thus, the torsional angles about the CO-CO bond are 180° in MeO-Aib-CO-CO-Aib-OMe (135), 175° in MeO-Leu-CO-CO-Leu-OMe (127) and 107° for MeO-Pro-CO-CO-Pro-OMe (133).

Thus, it appears from the available crystal structure data (Scheme C.10) that an unrestrained oxalamide group assumes a planar or nearly so trans conformation, even with bulky substituents on the end atoms except when unavoidable steric hindrance occurs when the nitrogen atom is part of a cyclic system such as the pyrrolidine ring in a proline residue. Here steric hindrance between the CO and the ring enforces a near orthogonal disposition of the COCO unit. The most important observation pertaining to the crystal X-ray studies is that retropeptides having a dihedral angle close to orthogonality can be designed and prepared and these are expected to have a bearing on rotamase activity and therefore immune suppression.



The bi-directional elongation of the core units, shown in Chart C.16, was accomplished by two broad strategies. They were either hydrolyzed to the corresponding acids and coupled with the appropriate partners using DCC/HOBt procedure or were transformed to the hydrazide and coupled by the azide procedure.

In Chart C.17 is presented nine examples pertaining to the hydrolysis of the core motif to the corresponding carboxylic acid. These compounds represent a class of important constructs in the sense that they could be used in the synthesis of a variety of retropeptides of significance in protein design and protein cross linking (Chart C.17).

HO-Gly-CO-CO-Gly-OH (145): (62%)

mp. : 247-248°C (lit.¹⁷³ mp. 250°C)

HO-Ala-CO-CO-Ala-OH (146): (77%)

mp. : 194-195°C (lit.¹⁷³ mp. 195-205°C)

HO-Leu-CO-CO-Leu-OH (148): (93%)

mp. : 160-161°C

ir : ν_{\max} (KBr) cm^{-1} : 3274, 1725, 1678, 1514, 1469.

ms : m/z: 317 (MH)⁺.

HO-Tyr-CO-CO-Tyr-OH (149): (87%)

mp. : 244-245°C (lit.¹⁷³ mp. 245-247°C)

HO-Trp-CO-CO-Trp-OH (150): (80%)

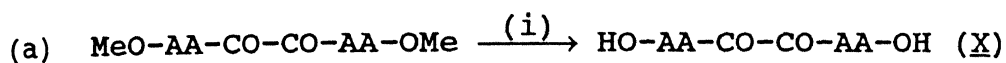
mp. : 215-217°C

ir : ν_{\max} (KBr) cm^{-1} : 3403 (NH), 3313 (NH), 3055 (br), 1736 (acid), 1664 (amide I), 1527 (amide II), 1455, 1424.

ms : m/z: 463 (MH)⁺.

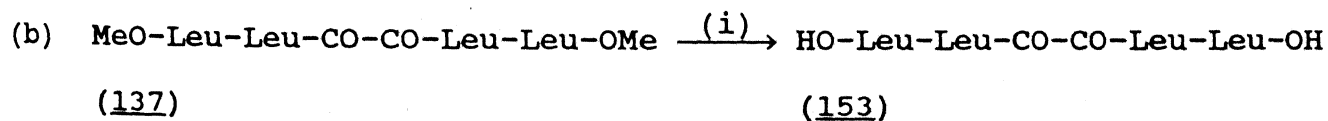
HO-Pro-CO-CO-Pro-OH (151): (76%)

mp. : 79-80°C

CHART C.17

AA = Amino Acid

AA (No.)	(X)
Gly (<u>124</u>)	HO-Gly-CO-CO-Gly-OH (<u>145</u>)
Ala (<u>125</u>)	HO-Ala-CO-CO-Ala-OH (<u>146</u>)
Leu (<u>127</u>)	HO-Leu-CO-CO-Leu-OH (<u>148</u>)
Tyr (<u>131</u>)	HO-Tyr-CO-CO-Tyr-OH (<u>149</u>)
Trp (<u>132</u>)	HO-Trp-CO-CO-Trp-OH (<u>150</u>)
Pro (<u>133</u>)	HO-Pro-CO-CO-Pro-OH (<u>151</u>)
Lys(N ^α Z) (<u>134</u>)	HO-Lys(N ^α Z)-CO-CO-Lys(N ^α Z)-OH (<u>155</u>)
Aib (<u>135</u>)	HO-Aib-CO-CO-Aib-OH (<u>147</u>)



(i) 2N NaOH-MeOH/0°/rt/4 h

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 2983 (br), 2591 (br), 1741 (acid), 1638 (br, amide I), 1513 (amide II), 1456, 1408, 1316.

HO-(N ^{α} Z)Lys-CO-CO-Lys(N ^{α} Z)-OH (155): (73%)

mp. : sticky solid

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3330 (br, NH), 2940 (br), 1685 (br, amide I), 1508 (br, amide II).

HO-Aib-CO-CO-Aib-OH (147): (79%)

mp. : 280-281°C (lit.¹⁷⁴ mp. 284°C)

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3297 (NH), 1740, 1656 (amide I), 1522 (amide II), 1470.

ms : m/z: 261 (MH)⁺.

HO-Leu-Leu-CO-CO-Leu-Leu-OH (153): (63%)

mp. : 153-154°C

An interesting discovery was made when the copper salts of these core retropeptido-mimetic oxalamide acids, readily prepared from treatment with $\text{Cu}(\text{NO}_3)_2$ or CuCO_3 , were found to harbor two copper atoms per substrate (Chart C.18).

(Gly-CO-CO-Gly) Cu_2 (156): (70%)

mp. : colour changes from blue to green at 260-270°C, changes to black at 320-325°C, does not melt.

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3200 (br, NH), 1620 (br), 1375, 1300, 1280.

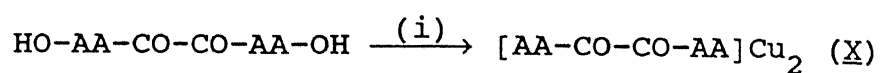
ms : m/z: 329 (M)⁺.

(Aib-CO-CO-Aib) Cu_2 (157): (95%)

mp. : colour changes from blue to dark green at 260-270°C, turns black at 320-325°C, does not melt upto 350°C.

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3363, 1712, 1644, 1562, 1500, 1466, 1421, 1325, 1182.

CHART C.18



AA = Amino Acid

AA (No.)	(X)
Gly (<u>145</u>)	[Gly-CO-CO-Gly]Cu ₂ (<u>156</u>)
Aib (<u>147</u>)	[Aib-CO-CO-Aib]Cu ₂ (<u>157</u>)
Leu (<u>148</u>)	[Leu-CO-CO-Leu]Cu ₂ (<u>158</u>)
Tyr (<u>149</u>)	[Tyr-CO-CO-Tyr]Cu ₂ (<u>159</u>)
Trp (<u>150</u>)	[Trp-CO-CO-Trp]Cu ₂ (<u>160</u>)
Pro (<u>151</u>)	[Pro-CO-CO-Pro]Cu ₂ (<u>161</u>)
(N ^α Z) Lys (<u>155</u>)	[(N ^α Z) Lys-CO-CO-Lys(N ^α Z)]Cu ₂ (<u>162</u>)

(i) MeOH-Aq. basic Cu^{II} carbonate/reflux 0.25 h

ms : m/z: 321 (M - Cu)⁺.

(Leu-CO-CO-Leu)Cu₂ (158): (90%)

mp. : colour changes to black at 260-270°C, does not melt upto 310°C.

ir : ν_{\max} (KBr)cm⁻¹: 3385, 3302 (br), 1665, 1512, 1468.

(Tyr-CO-CO-Tyr)Cu₂ (159): (91%)

mp. : colour changes to black at 245-250°C, does not melt upto 310°C.

ir : ν_{\max} (KBr)cm⁻¹: 3382 (br), 1658, 1588, 1544, 1513, 1442, 1422, 1364, 1314, 1277.

ms : m/z: 541 (M)⁺.

(Trp-CO-CO-Trp)Cu₂ (160): (98%).

mp. : starts turning black at 190°C, becomes totally black at 200°C and decomposes at 255-260°C.

ir : ν_{\max} (KBr)cm⁻¹: 3397 (br), 1654 (br), 1506, 1456, 1418, 1341, 743.

ms : m/z: 587 (M)⁺.

(Pro-CO-CO-Pro)Cu₂ (161): (90%)

mp. : colour changes to black at 205-8°C and melts at 232-234°C.

ir : ν_{\max} (KBr)cm⁻¹: 2925 (br), 1741, 1617, 1513, 1455, 1407, 1346, 1215, 1184.

ms : m/z: 444 (M⁺ + 2H₂O - 1)⁺.

(N^αZ-Lys-CO-CO-Lys-N^αZ)Cu₂ (162): (50%).

mp. : turns black at 250-260°C, does not melt upto 335°C.

ir : ν_{\max} (KBr)cm⁻¹: 3540, 3450, 1670 (br), 1410, 1370, 800.

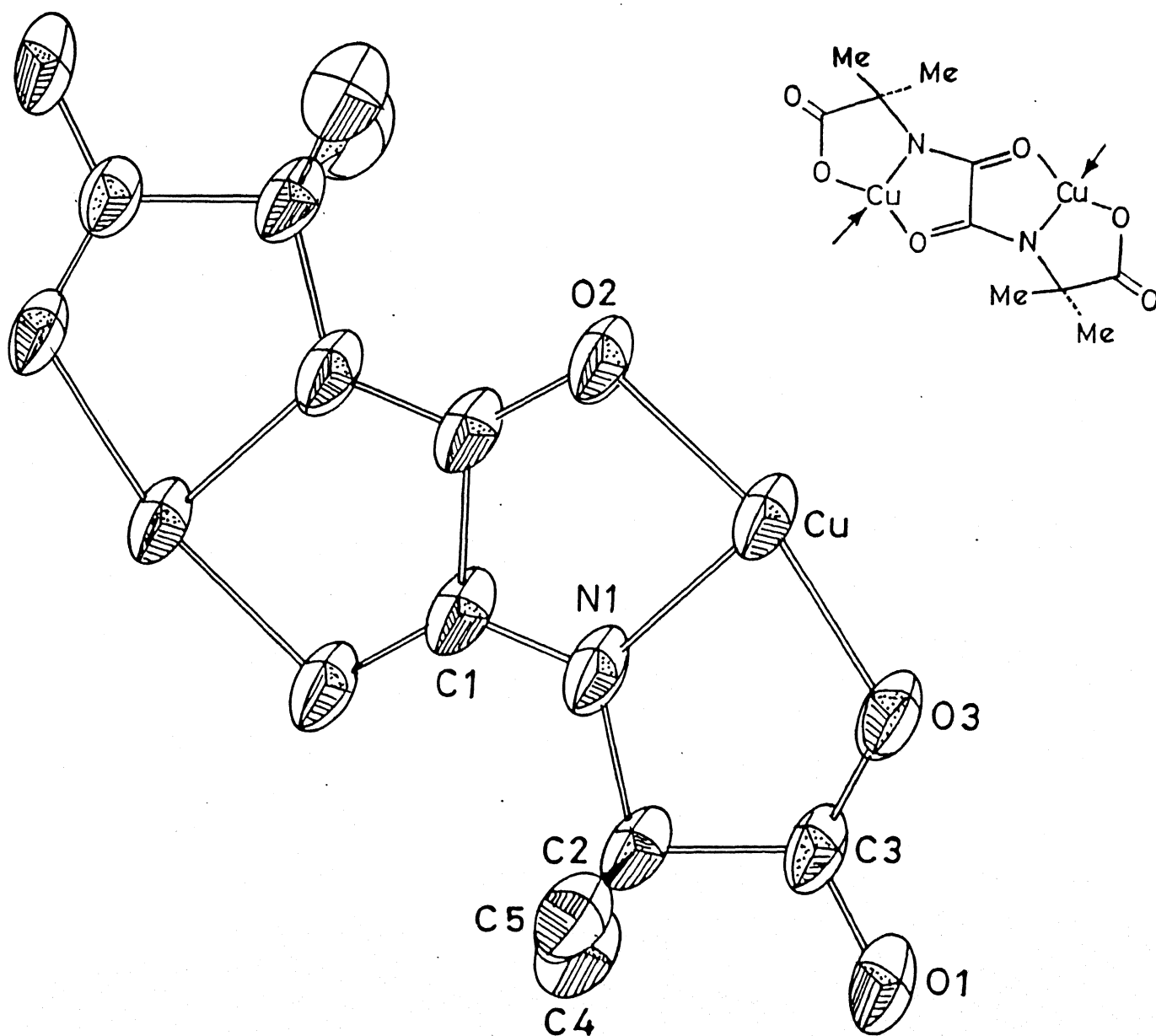
ms : m/z: 740 (M + 1)⁺.

The core diacids, shown in Chart C.17, by and large are C_2 symmetric around the oxalamide core (*vide supra*). The presence of two Cu atoms per unit can be best rationalized on the basis of each half of the unit harboring a Cu atom, which would mean the active participation of the C_2 disposed oxalamide grouping. This expectation was unequivocally demonstrated with the X-ray crystal structure of $(\text{Aib-CO-CO-Aib})\text{Cu}_2$ (157).

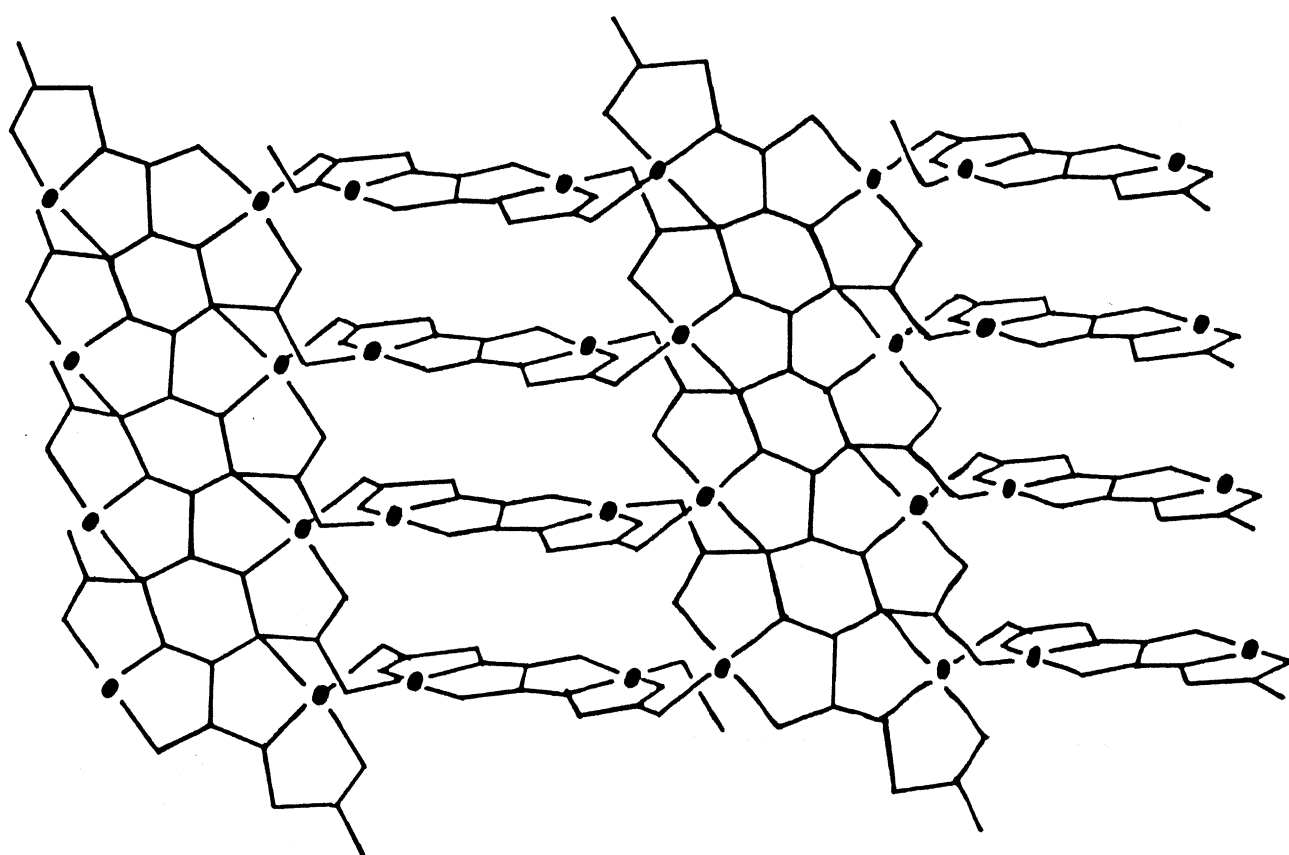
Thus, the X-ray crystal structure of the complex derived from $\text{HO-Aib-CO-CO-Aib-OH}$ (147) showed that the complex is a $(\text{Cu}_2\text{L})_n$ cluster in the solid state possessing a highly organized array of binuclear molecular blocks which are cross linked to the other blocks of the same lane as well as to that of neighboring lanes by carboxylato bridges. The ORTEP diagram of a single molecular block presented in Scheme C.11 shows that there are two Cu atoms per block, locked in a dimeric fashion, with the dimer (constituting two symmetric halves of the block) having a centre of inversion at the biscarbonyl unit of the core oxalamide motif. Further, each Cu atom in the dimer is coordinated to one nitrogen and two oxygen atoms of the same half, the fourth ligand of the nearly square planar template is provided in an exquisite manner by a neighboring carbonyl oxygen which also performs the pivotal role in bringing about the supramolecular self-assembly (Scheme C.12).

To our knowledge, this is the first demonstration of a retro-bispeptide metal complex enforcing a macromolecular organization to structure ensemble of current interest in several interdisciplinary domains.

The charge distribution of the ambident carboxylic moiety is weighted in favour to preserve individual molecules. This is evident from the fact that when dissolved in DMF the supramolecular assembly undergoes dissociation to individual molecule wherein the fourth ligand site is occupied by the solvent. The EPR studies clearly show complete dissociation to the individual molecules in DMF.

Scheme C.11

Scheme C.12



The EPR of the copper complexes, summarized in Table C.2, show that they are nearly planar. It is very likely that most members of this class will have a cluster profile in the solid state as has been established for (Aib-CO-CO-Aib) Cu_2 .

The bi-directional elongation strategy of core carboxylic acid retropeptido-mimetic oxalamido motifs has been illustrated by DCC/HOBt mediated coupling with appropriate partners. The reactions proceed smoothly to provide elongated peptides in excellent yields (Chart C.19.a).

MeO-Ala-Ala-CO-CO-Ala-Ala-OMe (136): (63%)

mp. : 216-217°C

ir : ν_{max} (KBr) cm^{-1} : 3318 (NH), 3271 (NH), 1737 (ester), 1641 (amide I), 1521 (amide II), 1506.

nmr : δ [400 MHz, $(\text{CD}_3)_2\text{SO}$]: 1.26 (12H, m, Ala $\text{CH}_3 \times 4$), 3.72 (6H, s, $\text{COOCH}_3 \times 2$), 4.26 (4H, m, Ala $\text{C}^\alpha\text{H} \times 4$), 8.48 (4H, m, exchangeable, Ala $\text{NH} \times 4$).

ms : m/z: 403 (MH) $^+$, 201 (M/2) $^+$.

anal: Found: C, 48.13; H, 6.74; N, 13.83 %

Calc. for $\text{C}_{16}\text{H}_{26}\text{N}_4\text{O}_8$: C, 47.76; H, 6.47; N, 13.93 %

$[\alpha]_{\text{D}}^{24}$: -56.36 (c, 0.33, MeOH).

MeO-Leu-Leu-CO-CO-Leu-Leu-OMe (137): (65%)

mp. : 200-201°C

ir : ν_{max} (KBr) cm^{-1} : 3261 (NH), 1747 (ester), 1651 (amide I), 1536 (amide II), 1510.

nmr : δ (CDCl_3): 0.90 (24H, brs, Leu $\text{CH}_3 \times 8$), 1.62 (12H, m, Leu $\text{C}^\beta\text{H}_2 \times 4$ + Leu $\text{C}^\gamma\text{H} \times 4$), 3.75 (6H, s, $\text{COOCH}_3 \times 2$), 4.50 (4H, m, Leu $\text{C}^\alpha\text{H} \times 4$), 6.59 (2H, d, $J=7.5$ Hz, exchangeable, $\text{NH} \times 2$), 7.93 (2H, d, $J=7.5$ Hz, exchangeable, $\text{NH} \times 2$).

ms : m/z: 571 (MH) $^+$, 285 (M/2) $^+$.

Table C.2

Retro-bispeptide copper complex : EPR and UV data

COMPLEX	EPR(-196°C)							UV	
	Solvent	A	g	g _⊥	g ₁	g ₂	g ₃	Solvent	$\lambda_{\max}^{\text{nm}}$ ($\epsilon, \text{Lmol}^{-1} \text{cm}^{-1}$)
(GOG)Cu ₂ (156)	H ₂ O:MeOH	125	2.29	2.06	---	---	---	MeOH	693 (101)
(BOB)Cu ₂ (157)	H ₂ O:MeOH	190	2.20	2.00	---	---	---	MeOH	688 (213)
(LOL)Cu ₂ (158)	H ₂ O:MeOH	130	2.36	2.08	---	---	---	MeOH	688 (125)
(YOY)Cu ₂ (159)	MeOH	150	2.26	--	2.077	2.057	2.035	MeOH	695 (184)
(WOW)Cu ₂ (160)	MeOH	127	2.37	2.07	---	---	---	MeOH	710 (85)
(POP)Cu ₂ (161)	H ₂ O:MeOH	165	2.29	2.06	---	---	---	MeOH	688 (132)
(KOK)Cu ₂ [*] (162)	MeOH	160	2.29	2.06	---	---	---	MeOH	691 (123)

* (KOK)Cu₂ = (N^αZ-KOK-N^αZ)Cu₂

One letter code used: G: Glycine; B: α -amino isobutyric acid; L: Leucine;
Y: Tyrosine; W: Tryptophan; P: Proline; K: Lysine.

anal: Found: C, 58.83; H, 8.68; N, 9.73 %

Calc. for $C_{28}H_{50}N_4O_8$: C, 58.95; H, 8.77; N, 9.82 %

$[\alpha]_D^{24}$: -64.93 (c, 1.66, MeOH).

MeO-Ser-Leu-CO-CO-Leu-Ser-OMe (139): (55%)

mp. : 205-206°C

ir : ν_{\max} (KBr) cm^{-1} : 3331 (NH), 1766, 1738 (ester), 1659 (amide I), 1523 (amide II).

nmr : δ (CDCl_3): 0.90 (12H, brs, Leu $\text{CH}_3 \times 4$), 1.75 (6H, m, Leu $\text{C}^\beta\text{H}_2 \times 2$ + Leu $\text{C}^\gamma\text{H} \times 2$), 3.78 (10H, s + m, $\text{COOCH}_3 \times 2$ + Ser $\text{C}^\beta\text{H}_2 \times 2$), 4.62 (2H, m, Ser $\text{C}^\alpha\text{H} \times 2$), 5.00 (2H, m, Leu $\text{C}^\alpha\text{H} \times 2$), 8.43 (4H, br, exchangeable, Ser $\text{NH} \times 2$ + Leu $\text{NH} \times 2$).

ms : m/z: 519 (MH)⁺, 259 (M/2)⁺.

anal: Found: C, 50.86; H, 7.38; N, 10.49 %

Calc. for $C_{22}H_{38}N_4O_{10}$: C, 50.96; H, 7.33; N, 10.81 %

$[\alpha]_D^{24}$: -32.85 (c, 0.70, MeOH).

MeO-Thr-Leu-CO-CO-Leu-Thr-OMe (140): (80%)

mp. : 197-198°C

ir : ν_{\max} (KBr) cm^{-1} : 3326 (NH), 1741 (ester), 1654 (amide I), 1626 (amide I), 1575 (amide II), 1522 (amide II).

nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 0.96 (12H, d, J=5.0 Hz, Leu $\text{CH}_3 \times 4$), 1.12 (6H, d, J=6.0 Hz, Thr $\text{CH}_3 \times 2$), 1.68 (6H, m, Leu $\text{C}^\beta\text{H}_2 \times 2$ + Leu $\text{C}^\gamma\text{H} \times 2$), 3.71 (6H, s, $\text{COOCH}_3 \times 2$), 4.14-4.80 (6H, m, Thr $\text{C}^\alpha\text{H} \times 2$ + Thr $\text{C}^\beta\text{H} \times 2$ + Leu $\text{C}^\alpha\text{H} \times 2$), 5.46 (2H, d, J=7.5 Hz, exchangeable, Thr $\text{OH} \times 2$), 8.06 (2H, d, J=7.5 Hz, exchangeable, $\text{NH} \times 2$), 8.56 (2H, d, J=7.5 Hz, exchangeable, $\text{NH} \times 2$).

ms : m/z: 547 (MH)⁺, 273 (M/2)⁺.

anal: Found: C, 52.39; H, 7.36; N, 10.69 %

Calc. for $C_{24}H_{42}N_4O_{10}$: C, 52.75; H, 7.69; N, 10.26 %

$[\alpha]_D^{24}$: -21.90 (c, 0.63, MeOH).

The iterative aspect of this novel bi-directional elongation has been demonstrated via further elongation of the first generation constructs. Thus, hydrolysis to HO-Leu-Leu-CO-CO-Leu-Leu-OH (153) followed by coupling with Leu-OMe and Ala-OMe afforded respectively MeO-Leu-Leu-Leu-CO-CO-Leu-Leu-Leu-OMe (142) and MeO-Ala-Leu-Leu-CO-CO-Leu-Leu-Ala-OMe (143) in good yields (Chart C.19.b).

MeO-Leu-Leu-Leu-CO-CO-Leu-Leu-Leu-OMe (142): (76%)

mp. : 283-284°C

ir : ν_{\max} (KBr) cm^{-1} : 3280 (NH), 1730 (ester), 1635 (amide I), 1528 (amide II), 1490.

nmr : δ (CDCl_3): 0.84 (36H, brs, Leu $\text{CH}_3 \times 12$), 1.62 (18H, m, Leu $\text{C}^\beta \text{H}_2 \times 6$ + Leu $\text{C}^\gamma \text{H} \times 6$), 3.68 (6H, s, $\text{COOCH}_3 \times 2$), 4.40 (3H, m, Leu $\text{C}^\alpha \text{H} \times 3$), 4.90 (3H, m, Leu $\text{C}^\alpha \text{H} \times 3$), 8.00-9.71 (6H, br, $\text{NH} \times 6$).

ms : m/z: 797 (MH)⁺.

anal: Found: C, 60.21; H, 9.18; N, 10.43 %

Calc. for $\text{C}_{40}\text{H}_{72}\text{N}_6\text{O}_{10}$: C, 60.30; H, 9.04; N, 10.55 %

$[\alpha]_D^{24}$: -108.0 (c, 0.43, CHCl_3).

MeO-Ala-Leu-Leu-CO-CO-Leu-Leu-Ala-OMe (143): (72%)

mp. : 156-157°C

ir : ν_{\max} (KBr) cm^{-1} : 3298 (NH), 3072, 1747 (ester), 1650 (amide I), 1546 (amide II), 1453.

nmr : δ (CDCl_3): 0.87 (24H, brs, Leu $\text{CH}_3 \times 8$), 1.31 (6H, d, $J=6.5$ Hz, Ala $\text{CH}_3 \times 2$), 1.62 (12H, m, Leu $\text{C}^\beta \text{H}_2 \times 4$ + Leu $\text{C}^\gamma \text{H} \times 4$), 3.75 (6H, s, $\text{COOCH}_3 \times 2$), 4.53 (6H, m, Ala $\text{C}^\alpha \text{H} \times 2$ + Leu $\text{C}^\alpha \text{H} \times 4$), 7.43 (4H, m, $\text{NH} \times 4$), 8.31 (2H, m, $\text{NH} \times 2$).

anal: Found: C, 57.36; H, 8.16; N, 11.64 %

Calc. for $\text{C}_{34}\text{H}_{60}\text{N}_6\text{O}_{10}$: C, 57.30; H, 8.43; N, 11.80 %

$[\alpha]_D^{24}$: -46.93 (c, 0.75, MeOH).

Similarly the azide coupling strategy for bi-directional elongation is illustrated in Chart C.20. As could be seen from here, the first generation construct (138) has been further processed by the same strategy to extended core retropeptido oxaloamido peptide (Chart C.20).

H_2NHN -Leu-CO-CO-Leu-NHNH₂ (152): (93%)

mp. : 219-220°C

MeO-Ala-Leu-CO-CO-Leu-Ala-OMe (138): (65%)

mp. : 184-185°C

ir : ν_{\max} (KBr) cm^{-1} : 3285 (NH), 1738 (ester), 1635 (amide I),
1505 (amide II).

H_2NHN -Ala-Leu-CO-CO-Leu-Ala-NHNH₂ (154): (78%)

mp. : 223-224°C

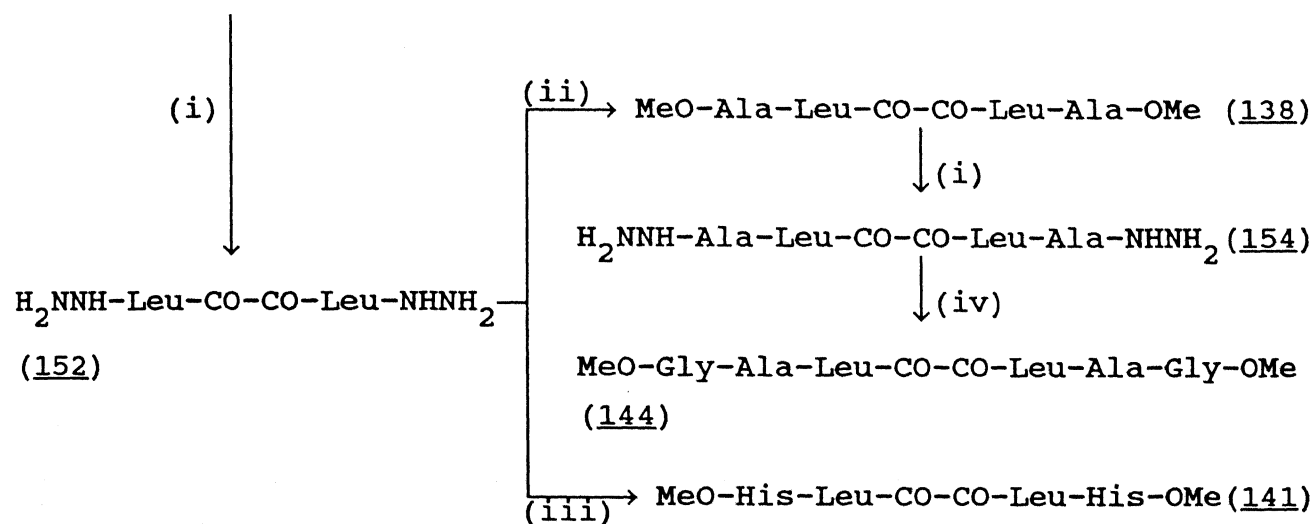
MeO-Gly-Ala-Leu-CO-CO-Leu-Ala-Gly-OMe (144): (30%)

mp. : crystals from methanol, 235-236°C

ir : ν_{\max} (KBr) cm^{-1} : 3280 (NH), 3045, 1739 (ester), 1628 (amide I), 1520 (amide II), 1500.

nmr : δ [400 MHz, CDCl₃ + 2% (CD₃)₂SO]: 0.95 (6H, d, J=5.0 Hz, Leu CH₃x2), 1.00 (6H, d, J=5.0 Hz, Leu CH₃x2), 1.31 (6H, d, J=6.5 Hz, Ala CH₃x2), 1.70 (6H, m, Leu C ^{β} H₂x2 + Leu C ^{γ} Hx2), 3.75 (6H, s, COOCH₃x2), 3.87 (2H, dd, Gly CHx2), 4.20 (2H, dd, Gly CHx2), 4.37 (2H, m, Leu C ^{α} Hx2), 4.58 (2H, m, Ala C ^{α} Hx2), 7.34 (2H, br, Gly NHx2), 7.47 (2H, d, J=7.5 Hz, Ala NHx2), 7.96 (2H, d, J=7.5 Hz, Leu NHx2).

¹³C nmr : δ [100.57 MHz, CDCl₃ + (CD₃)₂SO]: 17.42 (Leu CH₃), 21.36 (Ala CH₃), 22.75 (Leu C ^{β} H₂), 24.58 (Leu C ^{γ} H), 40.80 (Gly CH₂), 48.32 (COOCH₃), 52.03 (Leu C ^{α} H), 170.53, 171.04 (Leu CO, Ala CO), 172.45 (Gly CO).

CHART C.20(a) MeO-Leu-CO-CO-Leu-OMe (127)(i) $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$ -EtOH/rt/24 h;(ii) Aq. AcOH/6N HCl/ $\text{NaNO}_2/0^\circ$; H-Ala-OMe.HCl/ $\text{NEt}_3/\text{CH}_2\text{Cl}_2$;(iii) Aq. AcOH/6N HCl/ $\text{NaNO}_2/0^\circ$; H-His-OMe.2HCl/ $\text{NEt}_3/\text{CH}_2\text{Cl}_2$;(iv) Aq. AcOH/6N HCl/ $\text{NaNO}_2/0^\circ$; H-Gly-OMe.HCl/ $\text{NEt}_3/\text{CH}_2\text{Cl}_2$

ms : m/z: 600 (M)⁺.

anal: Found: C, 52.56; H, 6.87; N, 14.32 %

Calc. for C₂₆H₄₄N₆O₁₀: C, 52.00; H, 7.33; N, 14.00 %

MeO-His-Leu-CO-CO-Leu-His-OMe (141): (43%)

mp. : 124-126°C

ir : ν_{\max} (KBr) cm⁻¹: 3342 (NH), 1737 (ester), 1668 (amide I),
1547 (amide II), 1512 (amide II).

nmr : δ [400 MHz, CDCl₃ + (CD₃)₂SO]: 0.92 (12H, m, Leu CH₃x4),
1.66 (6H, m, Leu C ^{β} H₂x2 + Leu C ^{γ} Hx2), 3.10 (4H, m, His
C ^{β} H₂x2), 3.74 (6H, s, COOCH₃x2), 4.52 (2H, m, Leu C ^{α} Hx2),
4.75 (2H, m, His C ^{α} Hx2), 6.78 (2H, s, Imidazole ⁴Hx2), 7.50
(2H, s, Imidazole ²Hx2), 8.16 (2H, d, J=7.5 Hz, NHx2), 8.26
(2H, d, J=7.5 Hz, NHx2).

ms : m/z: 619 (MH)⁺, 309 (M/2)⁺.

anal: Found: C, 54.37; H, 6.48; N, 18.22 %

Calc. for C₂₈H₄₂N₈O₈: C, 54.37; H, 6.80; N, 18.12 %

[α]_D²⁴: -34.76 (c, 0.86, MeOH).

The symmetrical nature of the extended oxalo peptides, shown in Chart C.19 and Chart C.20, is reflected in their ¹H NMR and FAB mass spectra. Thus, the individual residues on either side of the core appear identical in the ¹H NMR spectra. Fortunately, interactions involving the oxalamido NH can be easily monitored because of the appearance of these NH protons as doublets at significantly lower fields (δ ~7.5-8.0) and their ready exchangeability. Each of the peptides exhibited in the FAB mass, peaks corresponding to MH⁺ which in many cases were the base peaks and also interestingly one's corresponding to (M/2)⁺.

The changing profile in structures arising from the elongation of the core motif have been probed by ¹H NMR spectroscopy. The correlation of the development of secondary structures as a function of bi-directional

elongation was the focus of such studies.

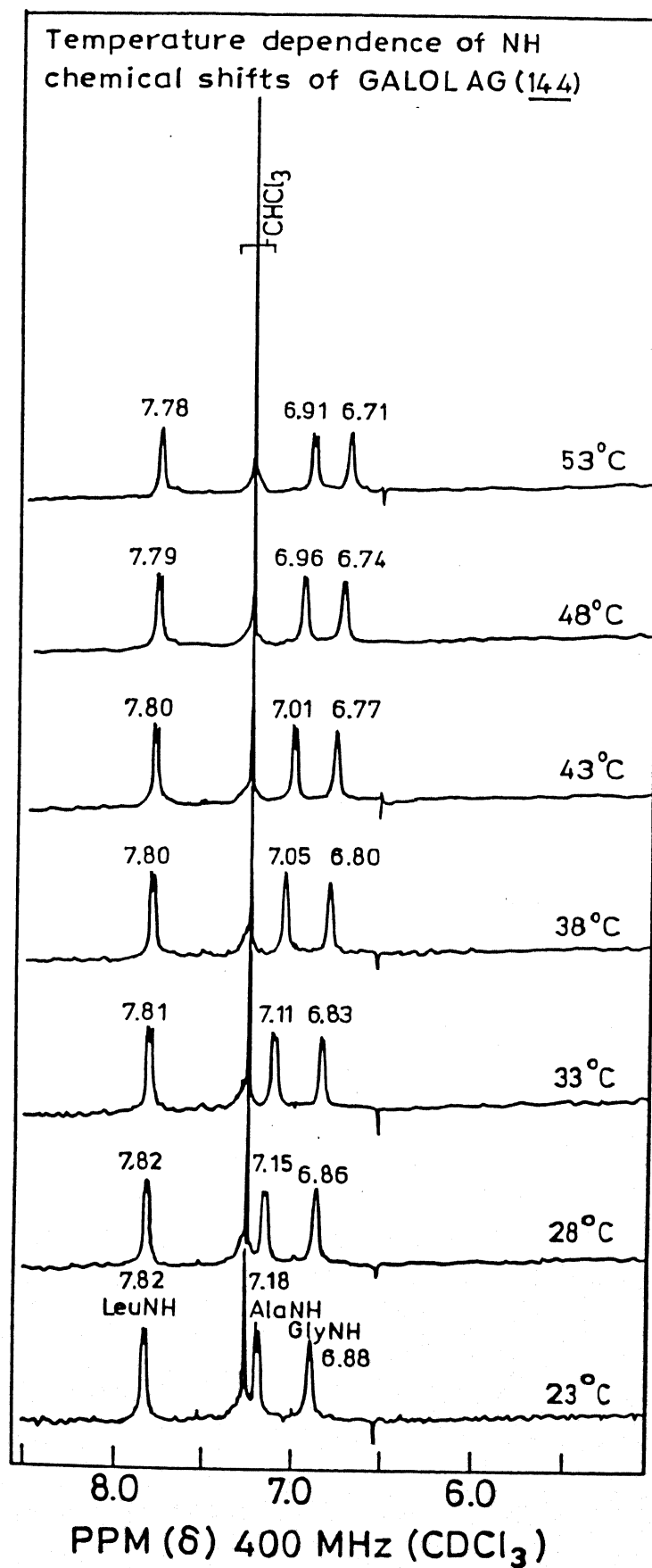
Temperature dependence of NH chemical shifts (VT studies) as observed in ^1H NMR spectra of MeO-Leu-Leu-CO-CO-Leu-Leu-OMe (137) showed that both the oxalamide and the Leu NH group of protons are solvent exposed and hence not involved in any intramolecular hydrogen bonding.

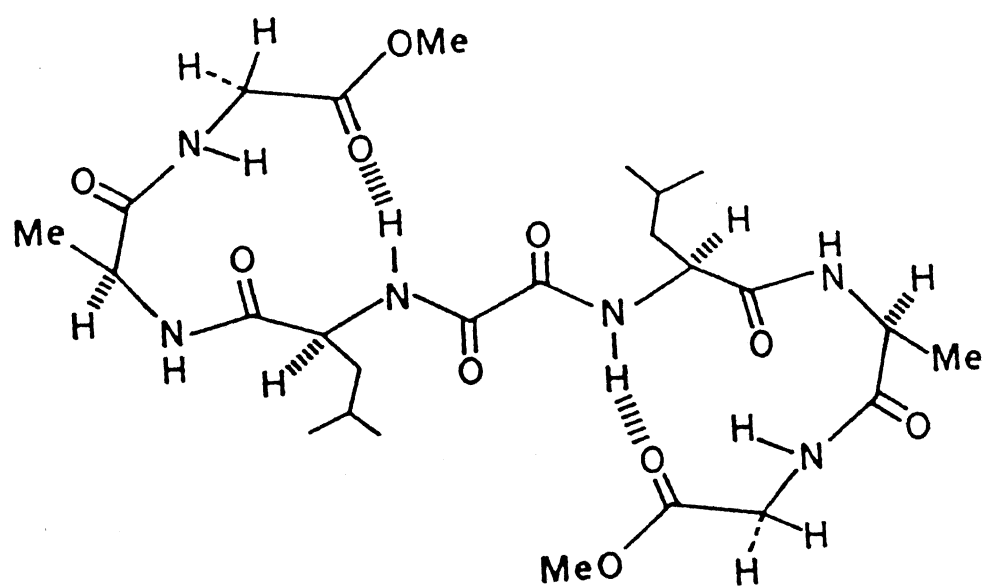
Bi-directional elongation with two residues resulted in the emergence of secondary structures. Thus, VT studies conducted in CDCl_3 between 296 and 326 $^\circ\text{K}$ with MeO-Gly-Ala-Leu-CO-CO-Leu-Ala-Gly-OMe (144) demonstrated for the Leu-NH protons — which forms an integral part of the oxalo peptide core — a $d\delta/dt$ values of -1.6 ppb K^{-1} , positively showing their involvement in intramolecular hydrogen bonding. Interestingly, the remaining two NH protons of Ala and Gly residues exhibited divergent behaviour exhibiting $d\delta/dt$ values respectively -9.00 and -5.60 ppb K^{-1} . These data show that while Ala NH protons are truly solvent exposed the nature of Gly NH remains ambiguous (Scheme C.13).

Based on the above, the novel C_2 symmetric structure (Scheme C.14) is proposed for MeO-Gly-Ala-Leu-CO-CO-Leu-Ala-Gly-OMe (144). The C_2 symmetric secondary structural motif arising from intramolecular hydrogen bonding, involving the Leu NH and Gly CO, is strongly supported by the chemical shift non-equivalence of the two Gly α protons due to restricted rotation around the ϕ , ψ torsion angles resulting in their appearance as two sets of double doublets at respectively δ 3.87 and 4.20.

The circular dichroism (CD spectra) of 144, in MeOH, demonstrates a distinct negative ellipticity at 231 nm supporting the envisaged secondary structure shown in Scheme C.14.

Regardless of fine details, the notion that the bi-directional elongation of the core motif can lead to secondary structural elements, seems secure. The systems and methodologies presented here will have ramification right across the protein domain, their potential in the modulation of protein function and protein design and their utility in

Scheme C.13

Scheme C.14

inter- or intra-strand cross linking, design of inhibitors, crafting of transition state mimics and the preparation of hormone antagonists could constitute some obvious options.

It may be recalled that when Ser-OMe was treated with oxalyl chloride, in place of the anticipated MeO-Ser-CO-CO-Ser-OMe, MeO- Δ Ala-CO-CO- Δ Ala-OMe (129) and MeO-Ser-CO-CO- Δ Ala-OMe (130) were formed. Since available methodologies for the generation of the Δ Ala units in a peptide environment is quite cumbersome, it was considered useful to explore the possibility of generation of the Δ Ala unit from serine precursors with oxalyl chloride-triethylamine. The ready formation of Bz- Δ Ala-OMe from Bz-Ser-OMe in 90% yields (Chart C.21.a) provided impetus to explore the process in detail.

In the event, as shown in Chart C.21.b to C.21.e eleven serine containing peptides on treatment with oxalyl chloride and triethylamine in dry CH_2Cl_2 at 0° for 2-4 hours, afforded the expected Δ Ala peptides in good to excellent yields. The Δ Ala unit present in the resulting chiral peptides could be easily identified by the presence of, in the NMR spectra, a non-exchangeable pair of singlets at δ ~5.5 to 7.0 and a broad exchangeable singlet at δ ~8.2 to 9.0. The facile formation of the dehydroalanine unit is rationalized on the basis of fragmentation of the initially formed Ser-O-oxalyl chloride (Scheme.C.15)

Bz- Δ Ala-OMe (163): (90%)

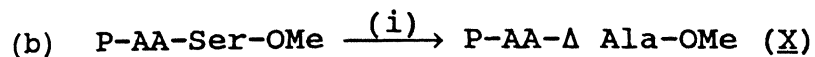
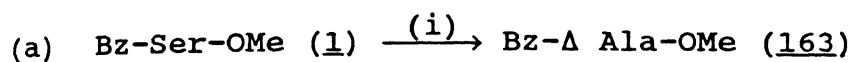
mp. : syrup

ir : ν_{max} (neat) cm^{-1} : 3323 (NH), 1776 (CONHC=CH₂), 1743 (ester), 1673 (br, amide I), 1530 (amide II), 1454.

nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 3.84 (3H, s, COOCH_3), 5.87, 6.50 (1H, 1H, s, s, =CH₂), 7.1-8.1 (5H, m, aromatic protons), 8.93 (1H, br, Δ Ala NH).

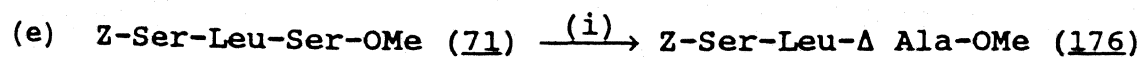
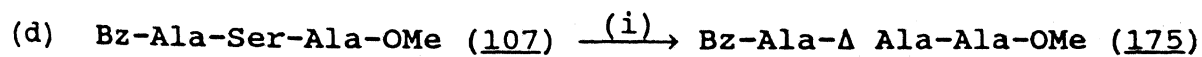
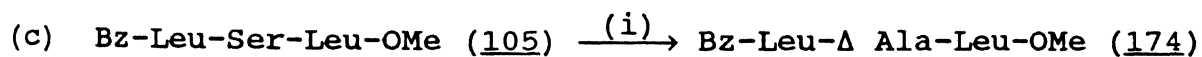
anal: Found: C, 64.44; H, 5.27; N, 6.83 %

CHART C.21

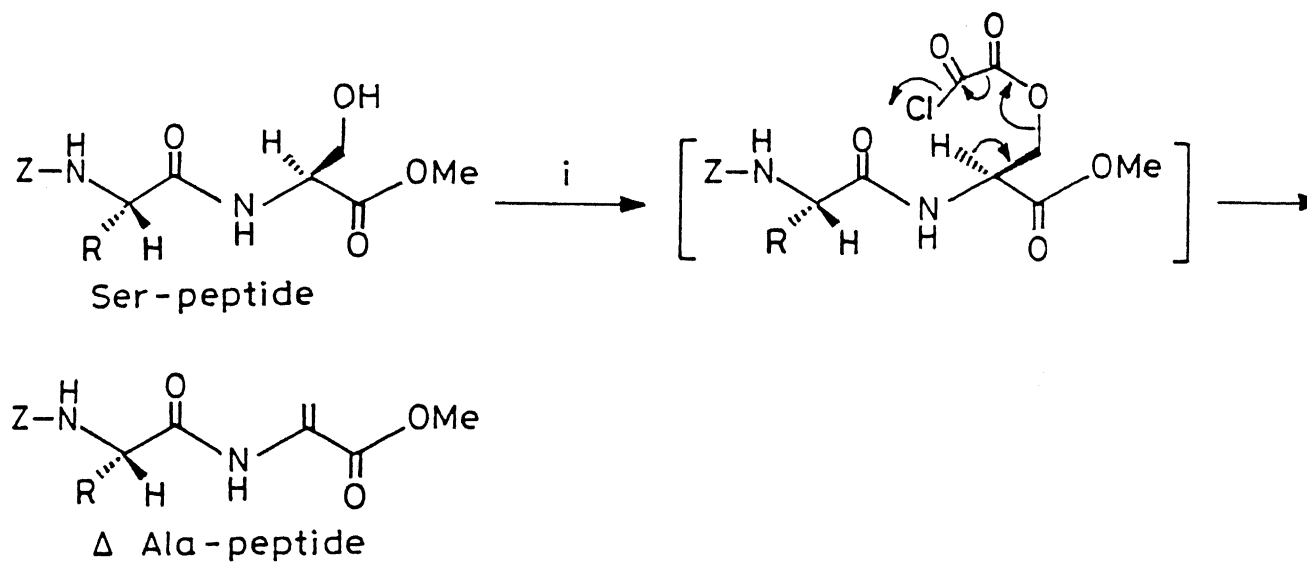


AA = Amino Acid

P	AA (No.)	(X)
Z	Gly (<u>5</u>)	Z-Gly- Δ Ala-OMe (<u>164</u>)
Bz	Ala (<u>9</u>)	Bz-Ala- Δ Ala-OMe (<u>165</u>)
Bz	Leu (<u>11</u>)	Bz-Leu- Δ Ala-OMe (<u>166</u>)
Z	Phe (<u>167</u>)	Z-Phe- Δ Ala-OMe (<u>168</u>)
Bz	Val (<u>27</u>)	Bz-Val- Δ Ala-OMe (<u>169</u>)
Bz	Pro (<u>29</u>)	Bz-Pro- Δ Ala-OMe (<u>170</u>)
Z	Pro (<u>171</u>)	Z-Pro- Δ Ala-OMe (<u>172</u>)
Z	Met (<u>25</u>)	Z-Met- Δ Ala-OMe (<u>173</u>)



(i) (COCl)₂/NEt₃/CH₂Cl₂ or THF or EtOAc/0°/4 h/rt

Scheme C.15

i: $(COCl)_2$, NEt_3 , CH_2Cl_2 , $0^\circ C$

Calc. for $C_{11}H_{11}NO_3$: C, 64.39; H, 5.37; N, 6.83 %

Z-Gly- Δ Ala-OMe (164): (40%)

mp. : syrup

ir : ν_{\max} (KBr) cm^{-1} : 3360 (NH), 1720 (br, ester), 1680 (amide I), 1510 (br, amide II), 1435.

nmr : δ (CDCl_3): 3.84 (3H, s, COOCH_3), 3.71-4.0 (2H, m, Gly CH_2), 5.15 (2H, s, Z CH_2), 5.59 (1H, m, Gly NH), 5.90, 6.59 (1H, 1H, s, s, $=\text{CH}_2$), 7.34 (5H, s, aromatic protons), 8.25 (1H, brs, Δ Ala NH).

ms : m/z: 293 (MH)⁺.

anal: Found: C, 57.43; H, 5.80; N, 9.36 %

Calc. for $C_{14}H_{16}N_2O_5$: C, 57.53; H, 5.48; N, 9.59 %

Bz-Ala- Δ Ala-OMe (165): (58%)

mp. : 110-115°C

ir : ν_{\max} (KBr) cm^{-1} : 3310 (NH), 1720 (ester), 1680 (amide I), 1623 (amide I), 1505 (br, amide II).

nmr : δ (60 MHz, CCl_4): 1.45 (3H, d, J=6.5 Hz, Ala CH_3), 3.78 (3H, s, COOCH_3), 4.75 (1H, m, Ala C^αH), 5.81, 6.53 (1H, 1H, s, s, $=\text{CH}_2$), 7.1-8.0 (6H, m, Ala NH + aromatic protons), 8.36 (1H, brs, Δ Ala NH).

ms : m/z: 277 (MH)⁺.

anal: Found: C, 60.49; H, 5.88; N, 10.26 %

Calc. for $C_{14}H_{16}N_2O_4$: C, 60.87; H, 5.80; N, 10.14 %

$[\alpha]_D^{23}$: -37.18 (c, 0.43, CHCl_3).

Bz-Leu- Δ Ala-OMe (166): (56%)

mp. : 55-56°C

ir : ν_{\max} (KBr) cm^{-1} : 3330 (NH), 1720 (ester), 1660 (amide I), 1625 (amide I), 1525 (amide II), 1475.

nmr : δ (60 MHz, CCl_4): 0.96 (6H, d, J=5.0 Hz, Leu $\text{CH}_3 \times 2$), 1.70

(3H, m, Leu C^βH₂ + Leu C^γH), 3.73 (3H, s, COOCH₃), 4.70 (1H, m, Leu C^αH), 5.83, 6.53 (1H, 1H, s, s, =CH₂), 7.13-7.90 (6H, m, Leu NH + aromatic protons), 8.43 (1H, brs, exchangeable with D₂O, Δ Ala NH).

ms : m/z: 319 (MH)⁺.

anal: Found: C, 64.22; H, 6.87; N, 8.58 %

Calc. for C₁₇H₂₂N₂O₄: C, 64.15; H, 6.92; N, 8.81 %

Z-Phe-Δ Ala-OMe (168): (58%)

mp. : syrup

ir : ν_{max}(neat)cm⁻¹: 3060, 1740 (br), 1643, 1500.

nmr : δ (CDCl₃): 3.40 (2H, m, Phe C^βH₂), 3.75 (3H, s, COOCH₃), 4.87 (1H, m, Phe C^αH), 5.41 (2H, s, Z CH₂), 5.43, 6.53 (1H, 1H, s, s, =CH₂), 7.03-7.65 (12H, m, Phe NH + Δ Ala NH + aromatic protons).

ms : m/z: 383 (MH)⁺.

anal: Found: C, 65.83; H, 5.58; N, 7.43 %

Calc. for C₂₁H₂₂N₂O₅: C, 65.97; H, 5.76; N, 7.33 %

[α]_D²³: +62.99 (c, 1.77, CHCl₃).

Bz-Val-Δ Ala-OMe (169): (48%)

mp. : syrup

ir : ν_{max}(neat)cm⁻¹: 3315, (br, NH), 1820, 1725 (br, ester), 1638 (amide I), 1520 (amide II).

nmr : δ (CDCl₃): 0.75-1.25 (6H, m, Val CH₃×2), 2.18 (1H, m, Val C^βH), 3.78 (3H, s, COOCH₃), 4.53 (1H, m, Val C^αH), 5.84, 6.53 (1H, 1H, s, s, =CH₂), 7.10-7.93 (6H, m, Val NH + aromatic protons), 8.25 (1H, brs, Δ Ala NH).

ms : m/z: 305 (M)⁺.

anal: Found: C, 62.83; H, 6.64; N, 9.11 %

Calc. for C₁₆H₂₁N₂O₄: C, 62.95; H, 6.89; N, 9.18 %

$[\alpha]_D^{23}$: +4.90 (c, 1.06, CHCl_3).

Bz-Pro- Δ Ala-OMe (170): (54%)

mp. : 110-111°C

ir : ν_{max} (KBr) cm^{-1} : 3390 (NH), 1730 (ester), 1685 (amide I), 1612 (amide I), 1510 (amide II).

nmr : δ (CDCl_3): 1.62-2.5 (4H, m, Pro C^βH_2 + Pro $\text{C}^\gamma\text{H}_2$), 3.53 (2H, t, Pro $\text{C}^\delta\text{H}_2$), 3.78 (3H, s, COOCH_3), 4.78 (1H, m, Pro C^αH), 5.84, 6.53 (1H, 1H, s, s, $=\text{CH}_2$), 7.21-7.59 (5H, m, aromatic protons), 8.96 (1H, brs, exchangeable with D_2O , Δ Ala NH).

ms : m/z: 303 (MH)⁺.

anal: Found: C, 63.42; H, 5.83; N, 9.18 %

Calc. for $\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_4$: C, 63.58; H, 5.96; N, 9.27 %

$[\alpha]_D^{23}$: -86.16 (c, 0.73, CHCl_3).

Z-Pro- Δ Ala-OMe (172): (58%)

mp. : gummy

nmr : δ (60 MHz, CDCl_3): 1.66-2.40 (4H, m, Pro C^βH_2 + Pro $\text{C}^\gamma\text{H}_2$), 3.43 (2H, t, Pro $\text{C}^\delta\text{H}_2$), 3.76 (3H, s, COOCH_3), 4.33 (1H, m, Pro C^αH), 5.10 (2H, s, Z CH_2), 5.70, 6.46 (1H, 1H, s, s, $=\text{CH}_2$), 7.20 (5H, s, aromatic protons), 8.73 (1H, brs, Δ Ala NH).

ms : m/z: 333 (MH)⁺.

Z-Met- Δ Ala-OMe (173): (62%)

mp. : syrup

ir : ν_{max} (KBr) cm^{-1} : 3360 (NH), 1810, 1718 (br, ester), 1505 (amide II), 1435.

nmr : δ (60 MHz, CCl_4): 2.03 (3H, s, Met S- CH_3), 2.46 (4H, m, Met C^βH_2 + Met $\text{C}^\gamma\text{H}_2$), 3.80 (3H, s, COOCH_3), 4.50 (1H, m, Met C^αH), 5.06 (2H, s, Z CH_2), 5.30, 5.80 (1H, 1H, s, s,

=CH₂), 6.56 (1H, brs, Met NH), 7.41 (5H, s, aromatic protons), 8.40 (1H, brs, Δ Ala NH).

ms : m/z: 367 (MH)⁺.

anal: Found: C, 55.44; H, 6.38; N, 7.29 %

Calc. for C₁₇H₂₂N₂O₅S: C, 55.74; H, 6.01; N, 7.65 %

Bz-Leu-Δ Ala-Leu-OMe (174): (30%)

mp. : syrup

ir : ν_{\max} (KBr)cm⁻¹: 3320 (NH), 1773, 1741 (ester), 1632 (br, amide I), 1525 (br, amide II).

nmr : δ (CDCl₃): 0.87 (12H, br, Leu CH₃x4), 1.65 (6H, m, Leu C^βH₂x2 + Leu C^γHx2), 3.71 (3H, s, COOCH₃), 4.09-5.21 (4H, m, Leu C^αHx2 + =CH₂), 7.00-8.06 (7H, m, Leu NHx2 + aromatic protons), 8.75 (1H, br, Δ Ala NH).

ms : m/z: 432 (MH)⁺.

anal: Found: C, 64.01; H, 7.34; N, 9.56 %

Calc. for C₂₃H₃₃N₃O₅: C, 64.04; H, 7.66; N, 9.74 %

[α]_D²³: -38.04 (c, 0.51, CHCl₃).

Bz-Ala-Δ Ala-Ala-OMe (175): (25%)

mp. : syrup

ir : ν_{\max} (KBr)cm⁻¹: 3345 (NH), 3290 (NH), 1740 (ester), 1690, 1623 (br, amide I), 1525 (amide II).

nmr : δ (CDCl₃): 1.53 (6H, m, Ala CH₃x2), 3.75 (3H, s, COOCH₃), 4.71 (2H, m, Ala C^αHx2), 5.40, 6.46 (1H, 1H, s, s, =CH₂), 6.84 (1H, m, Ala NH), 7.03-8.04 (6H, m, Ala NH + aromatic protons), 8.62 (1H, br, Δ Ala NH).

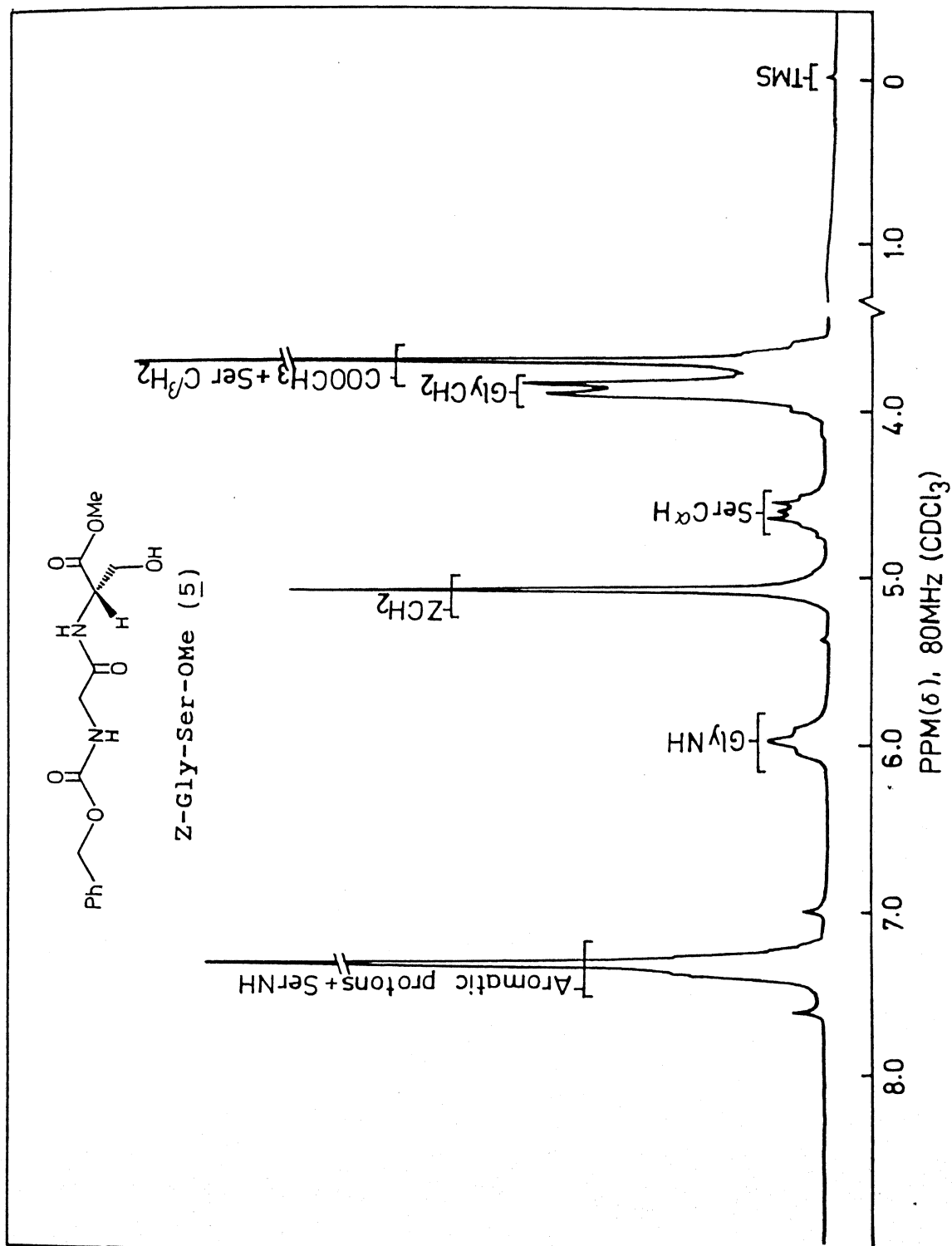
anal: Found: C, 59.11; H, 6.28; N, 12.11 %

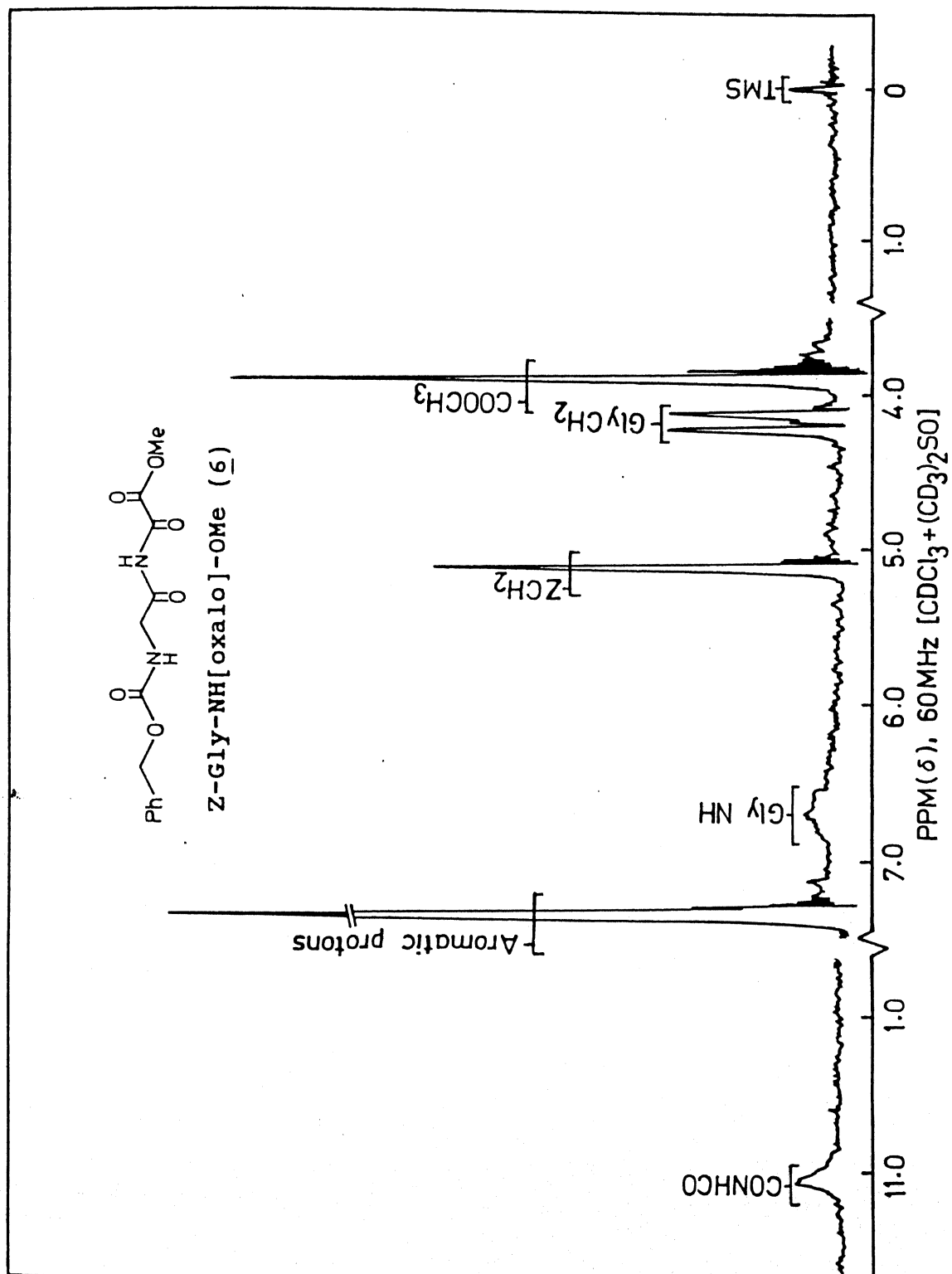
Calc. for C₁₇H₂₁N₃O₅: C, 58.79; H, 6.05; N, 12.10 %

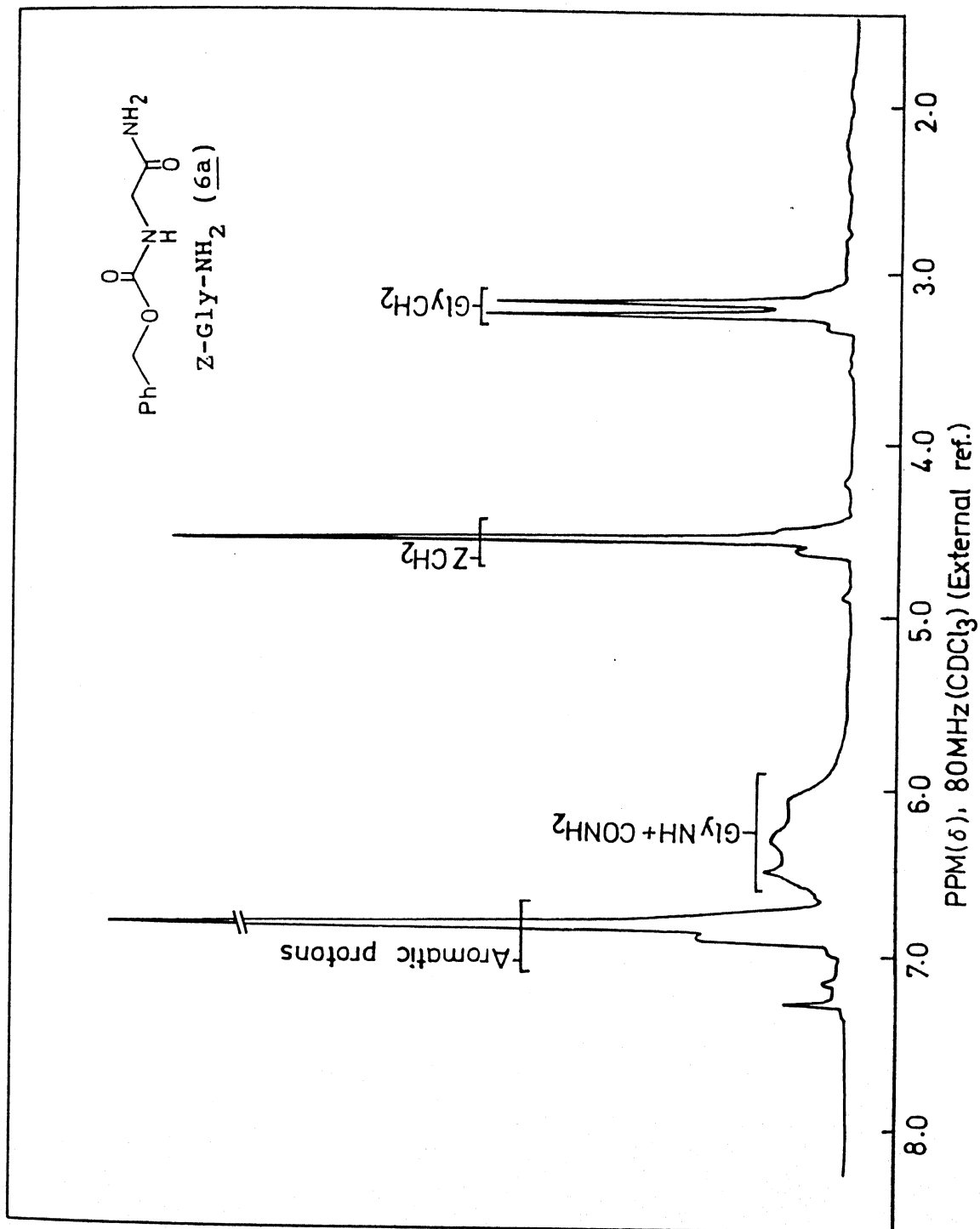
?-Ser-Leu-Δ Ala-OMe (176): (30%)

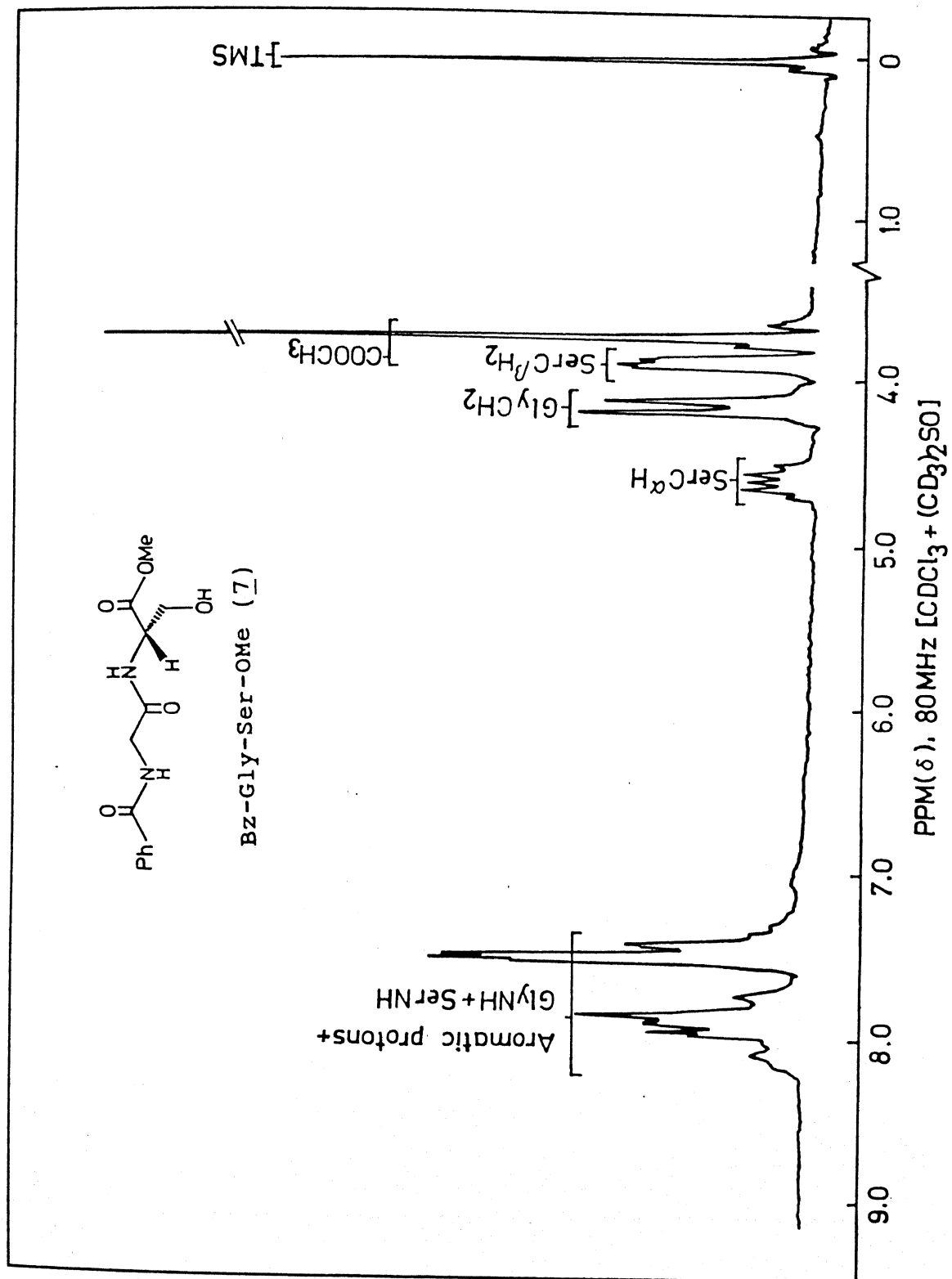
mp. : syrup

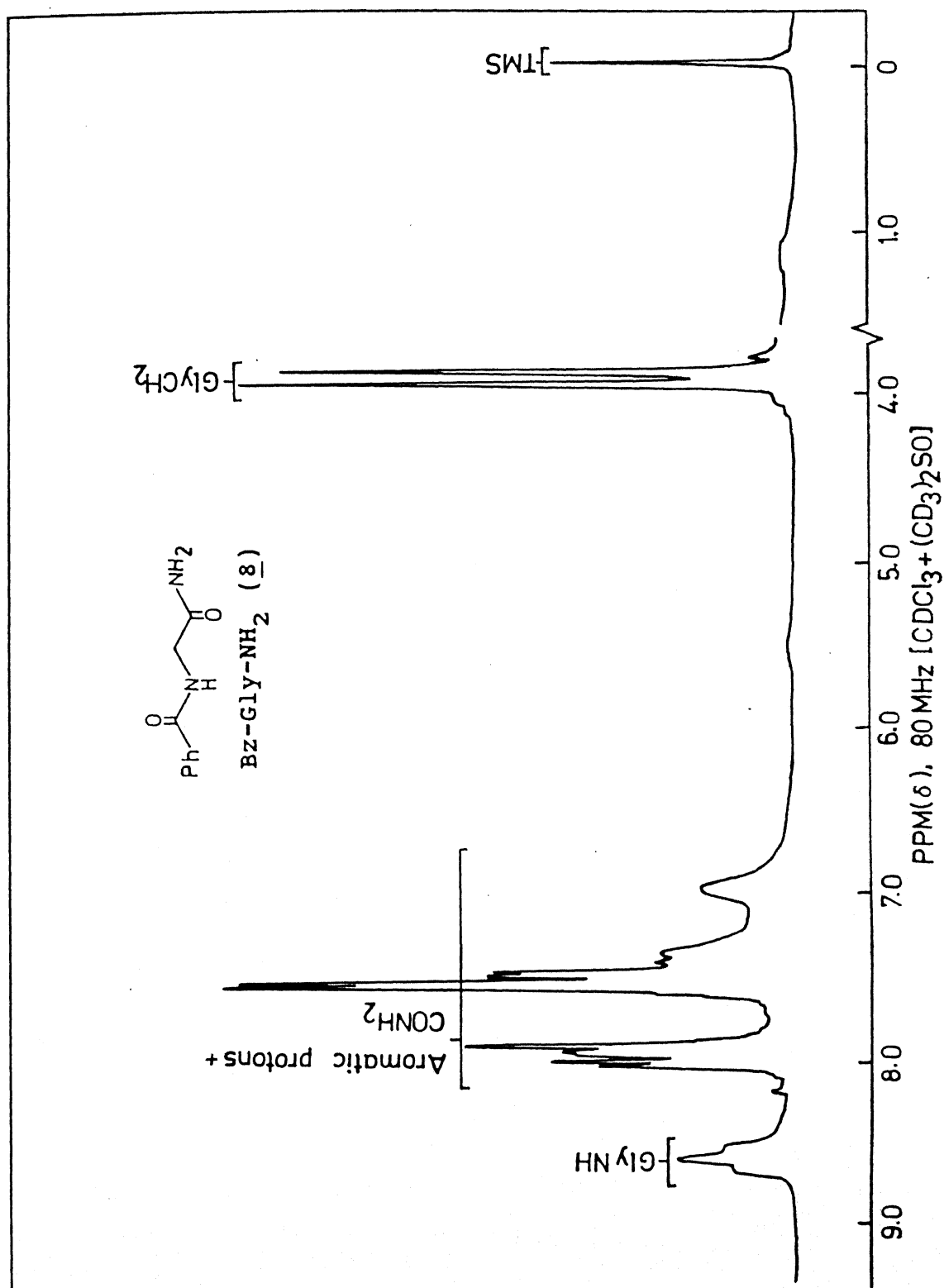
D. SPECTRA

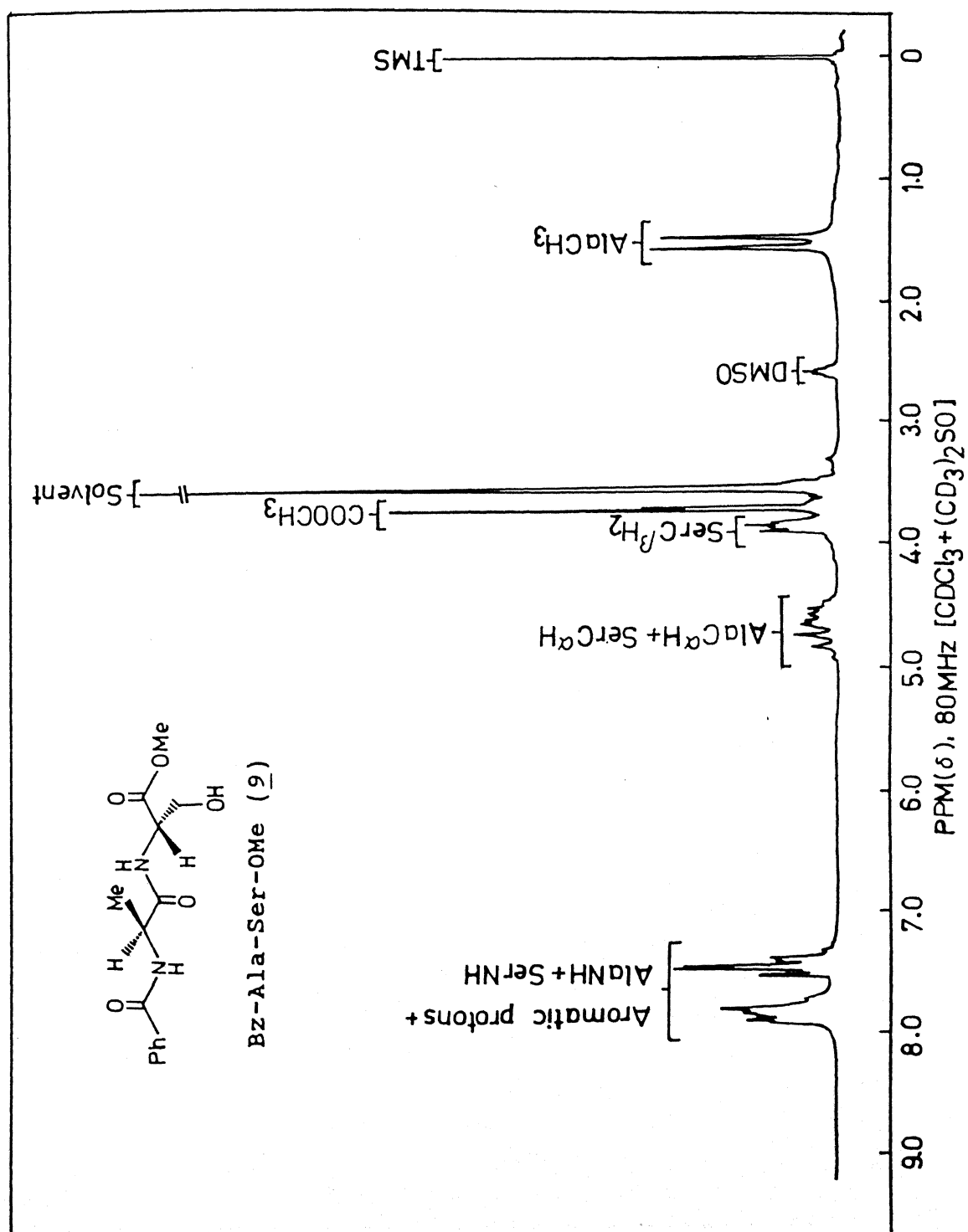


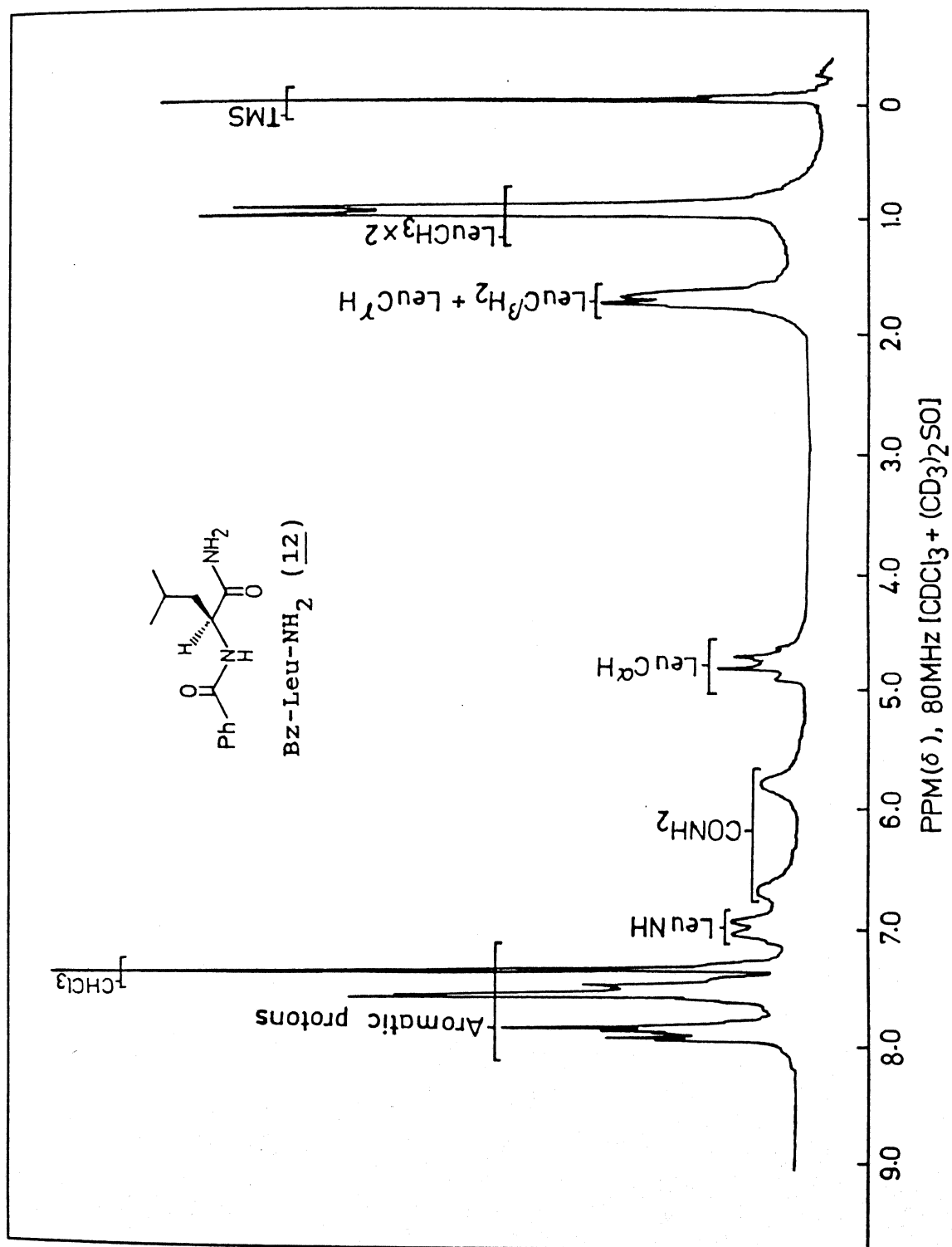


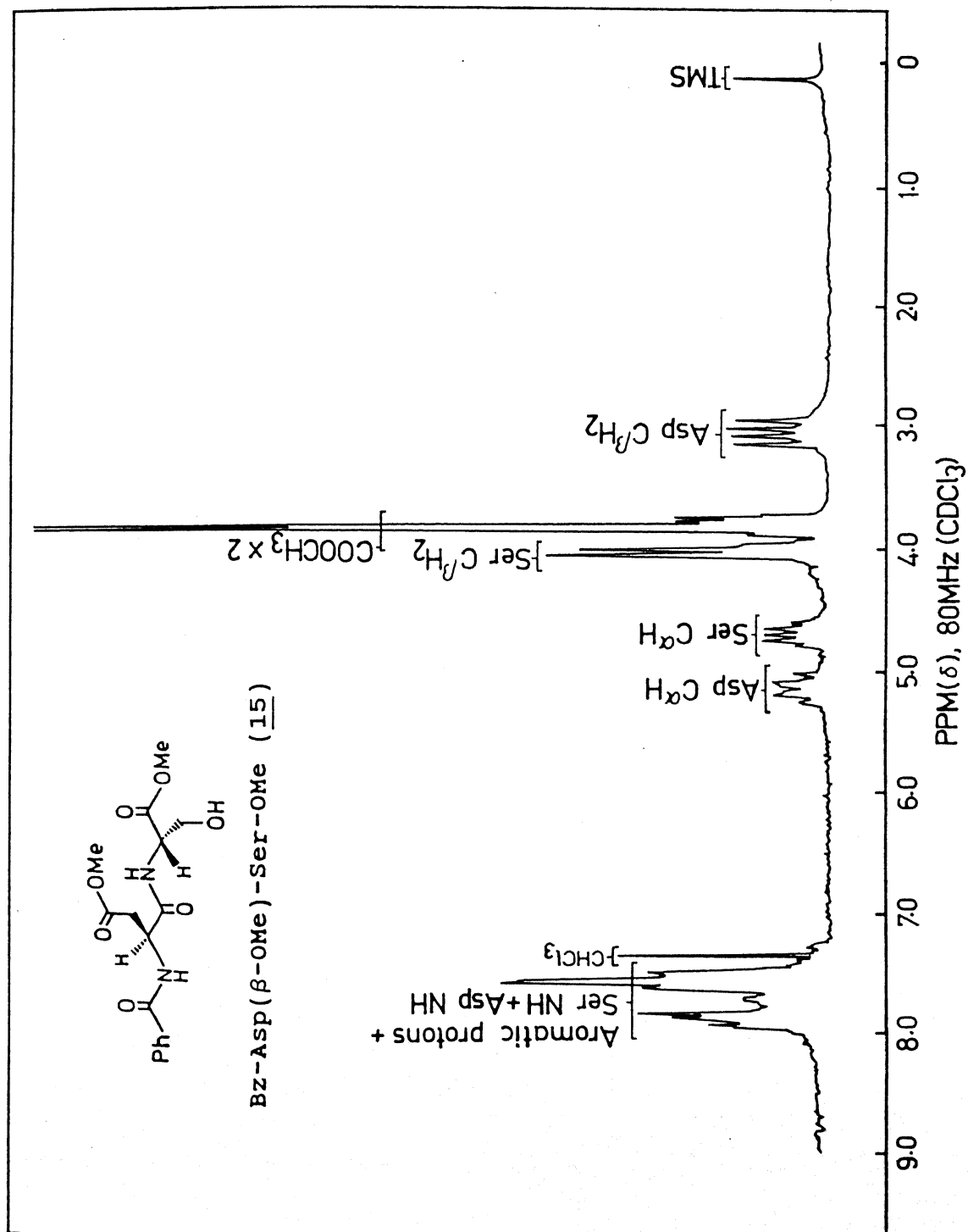


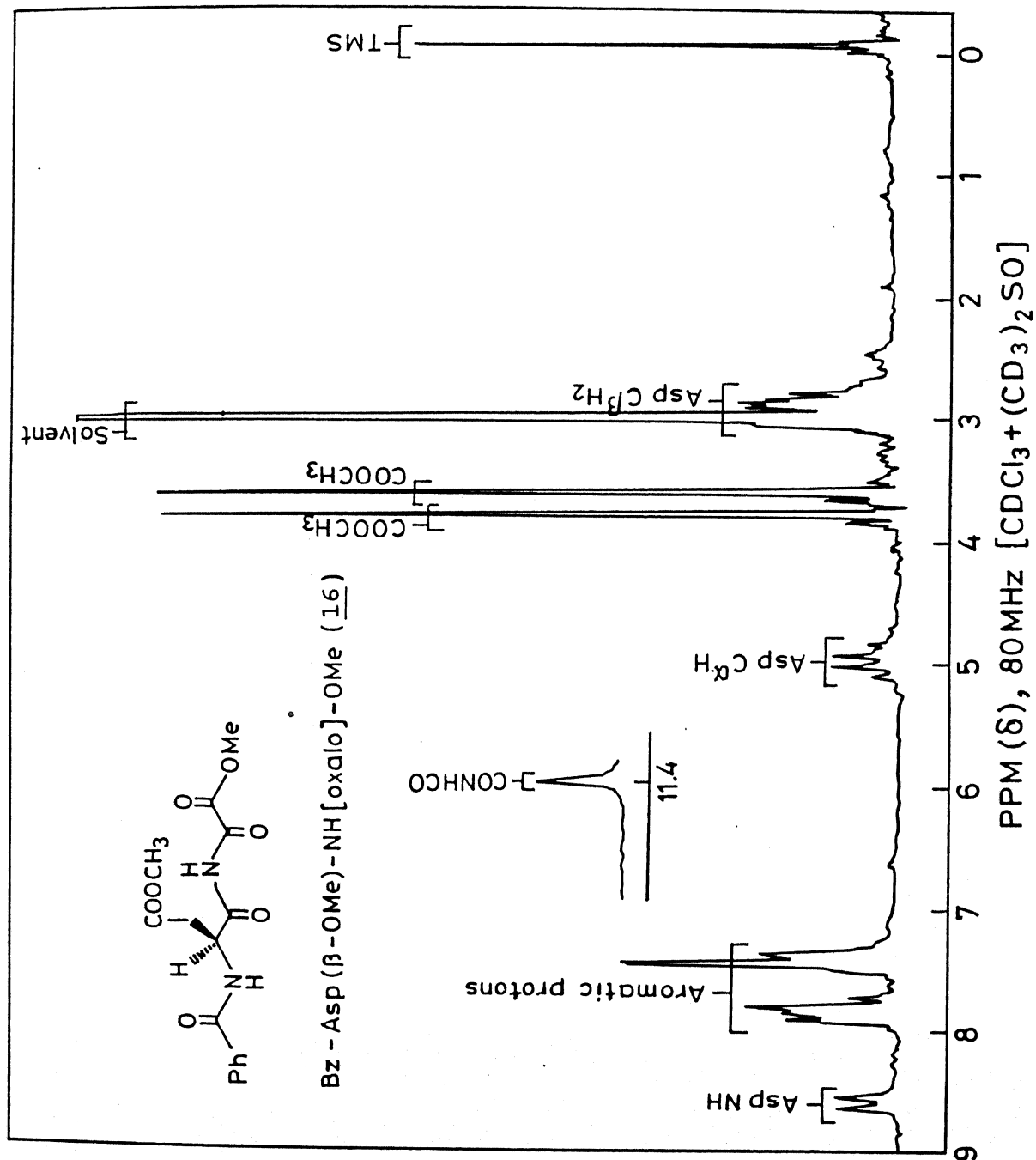


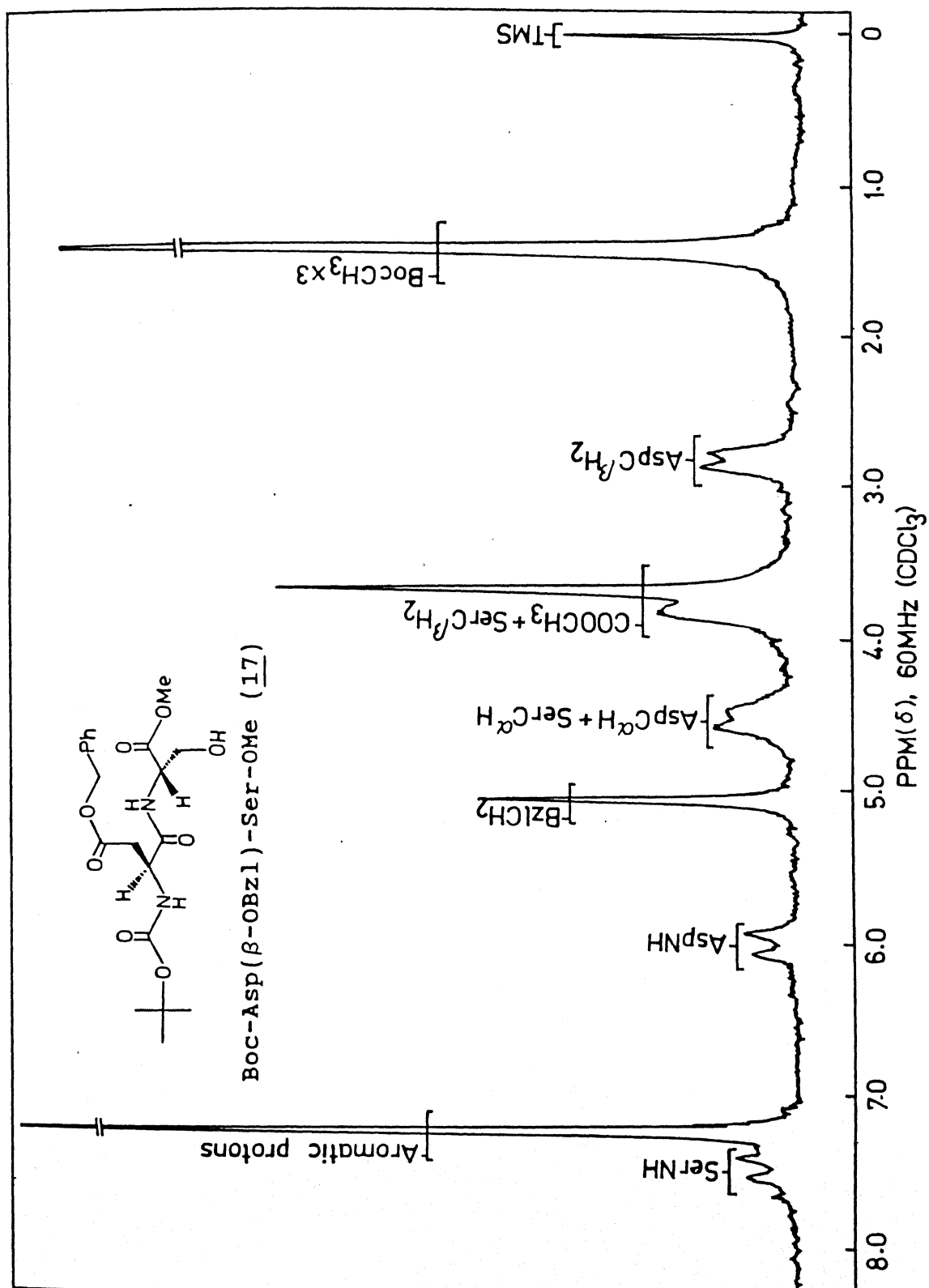


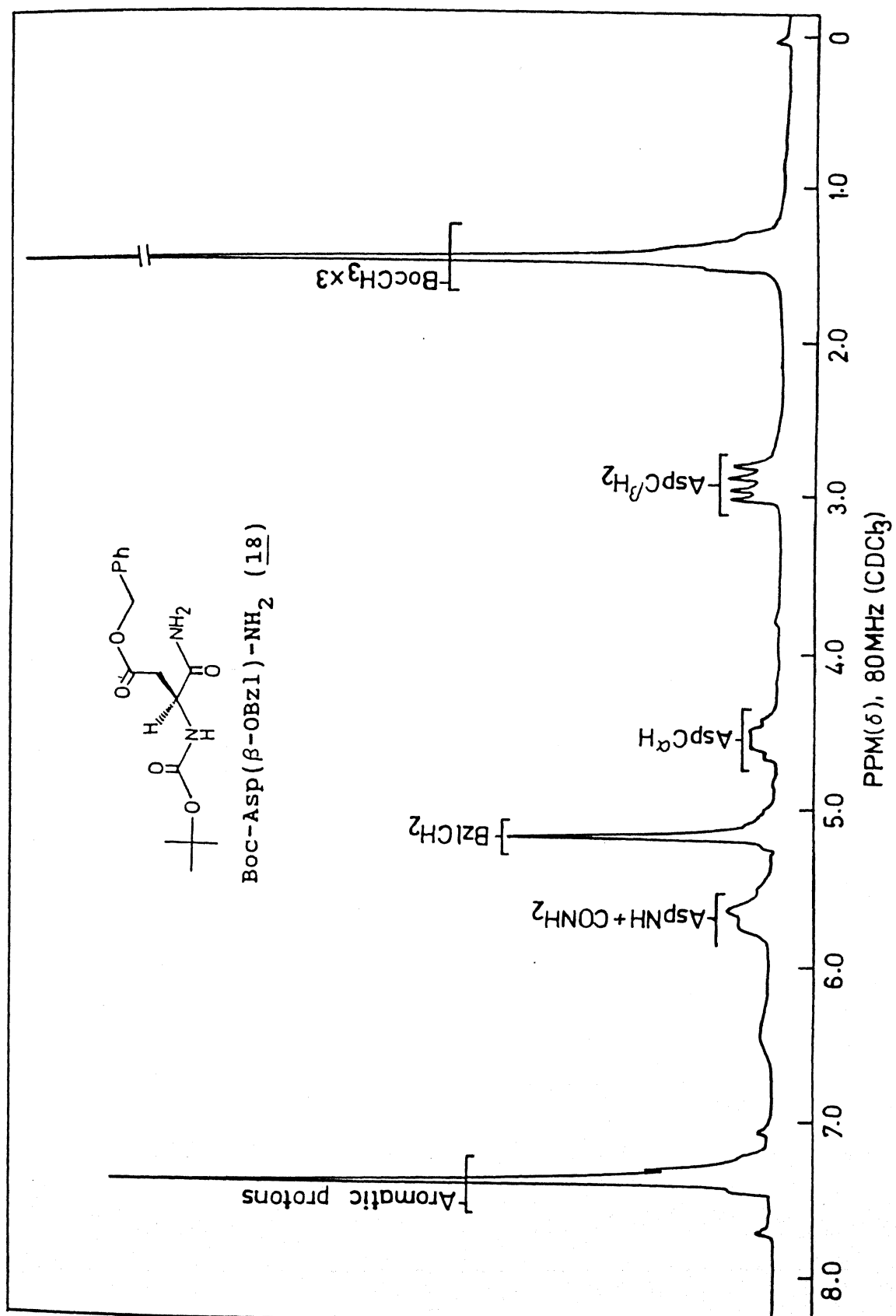


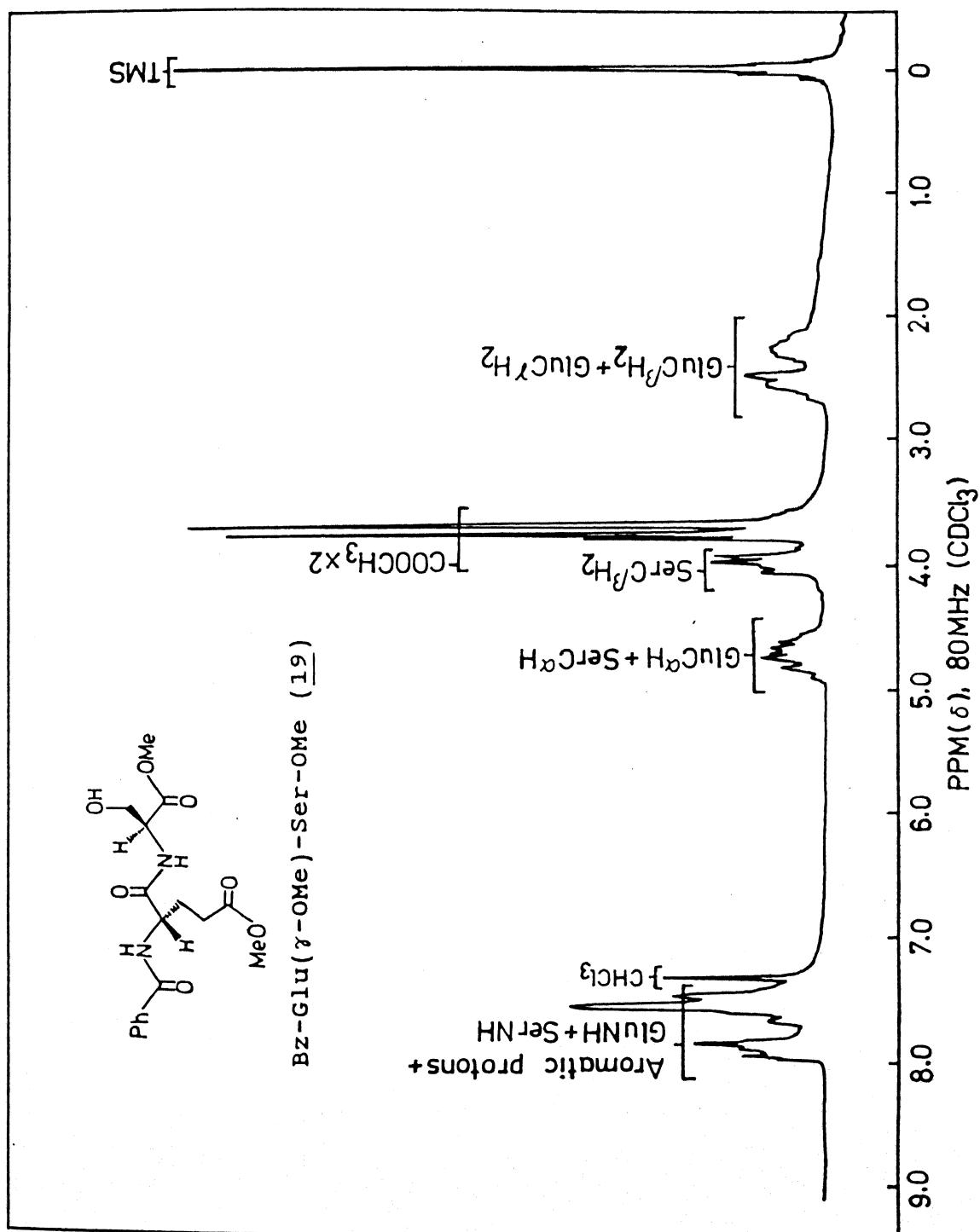


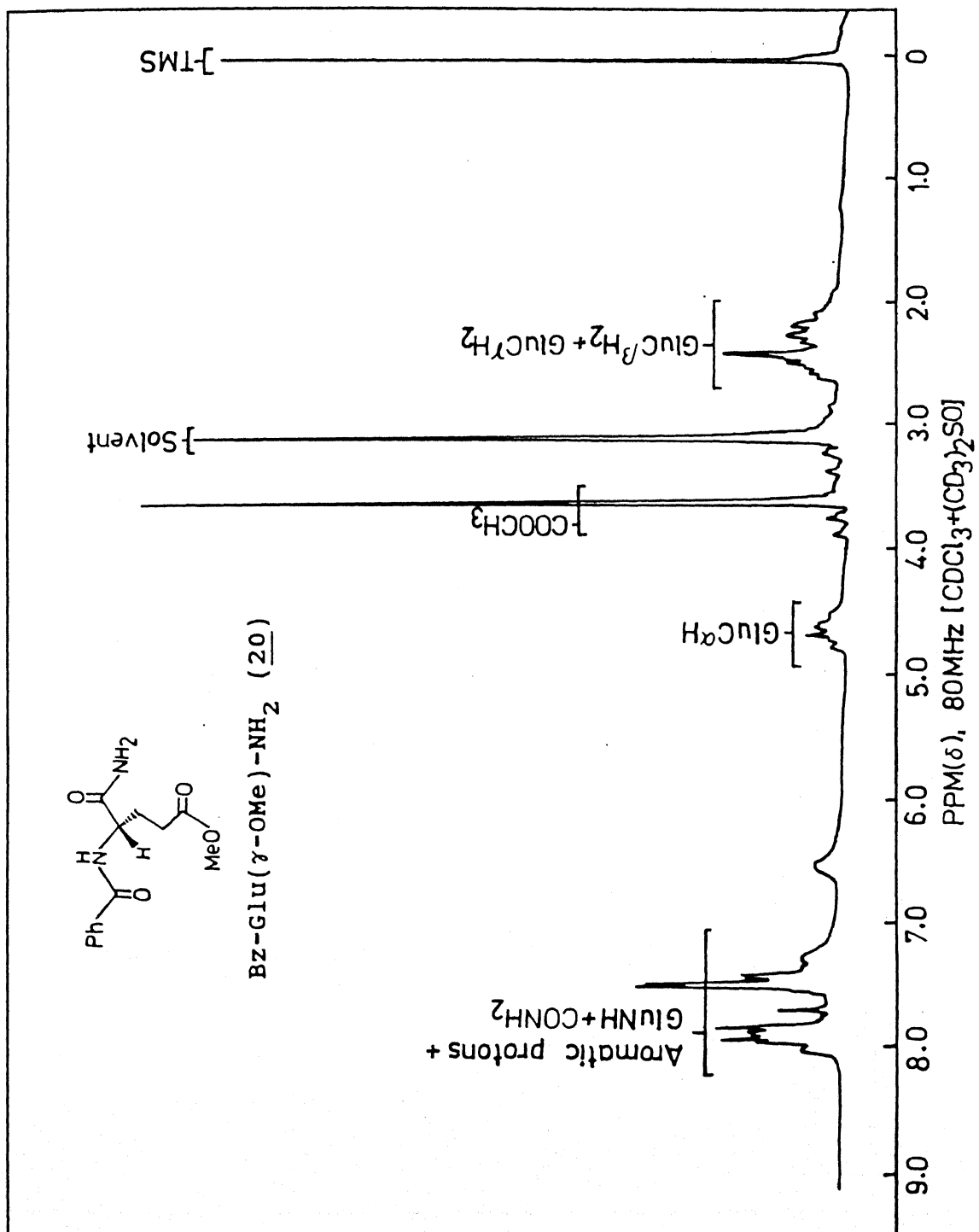


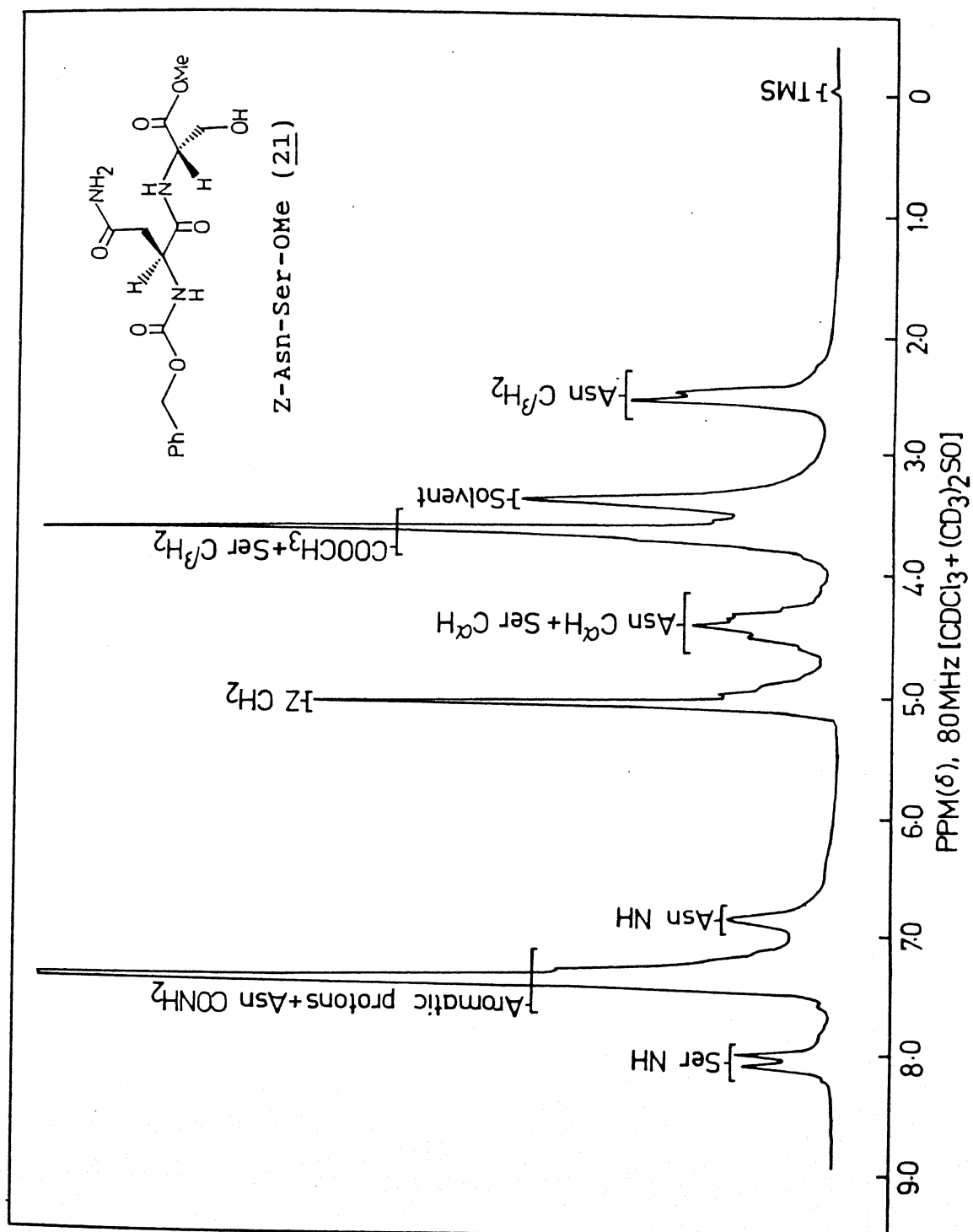


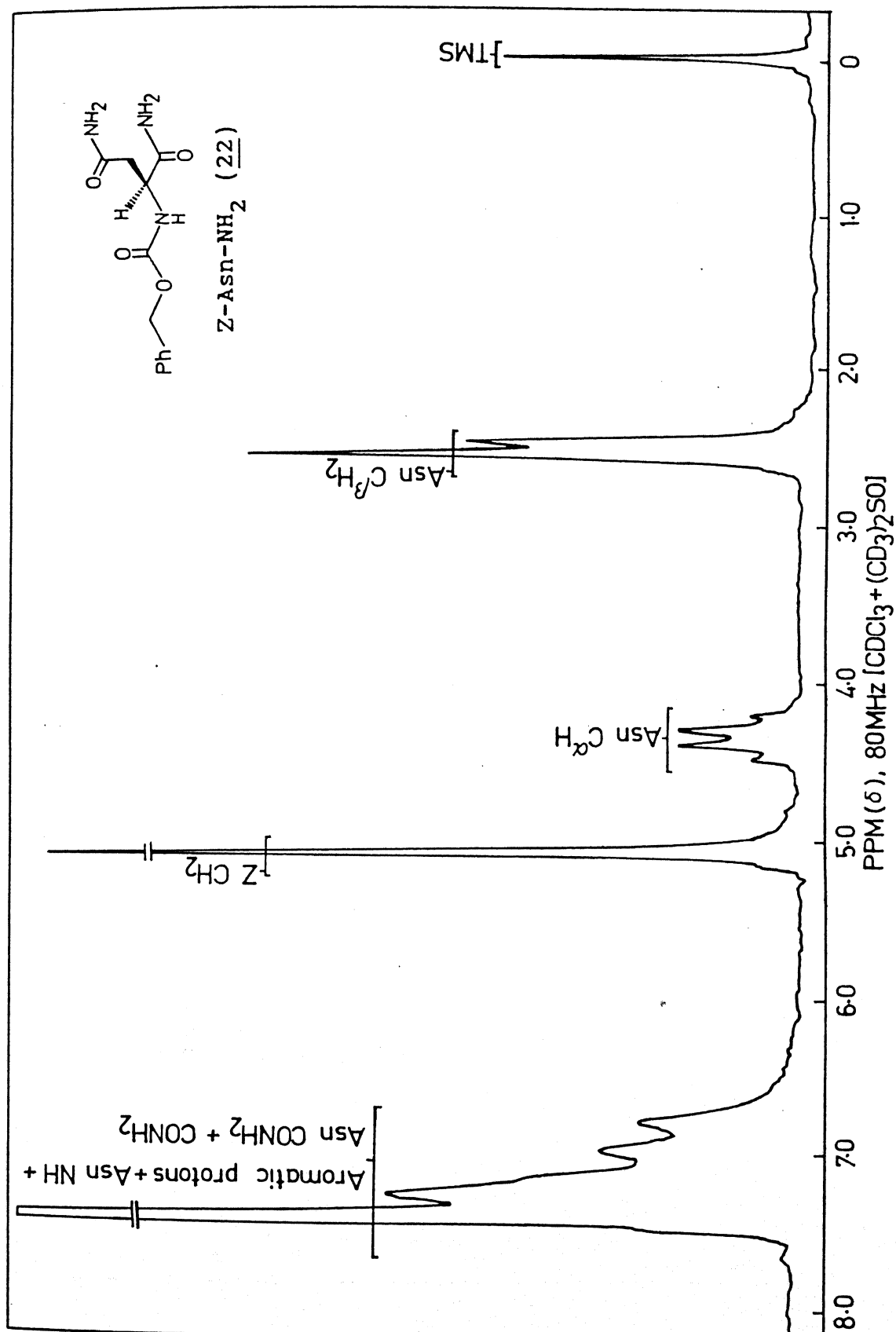


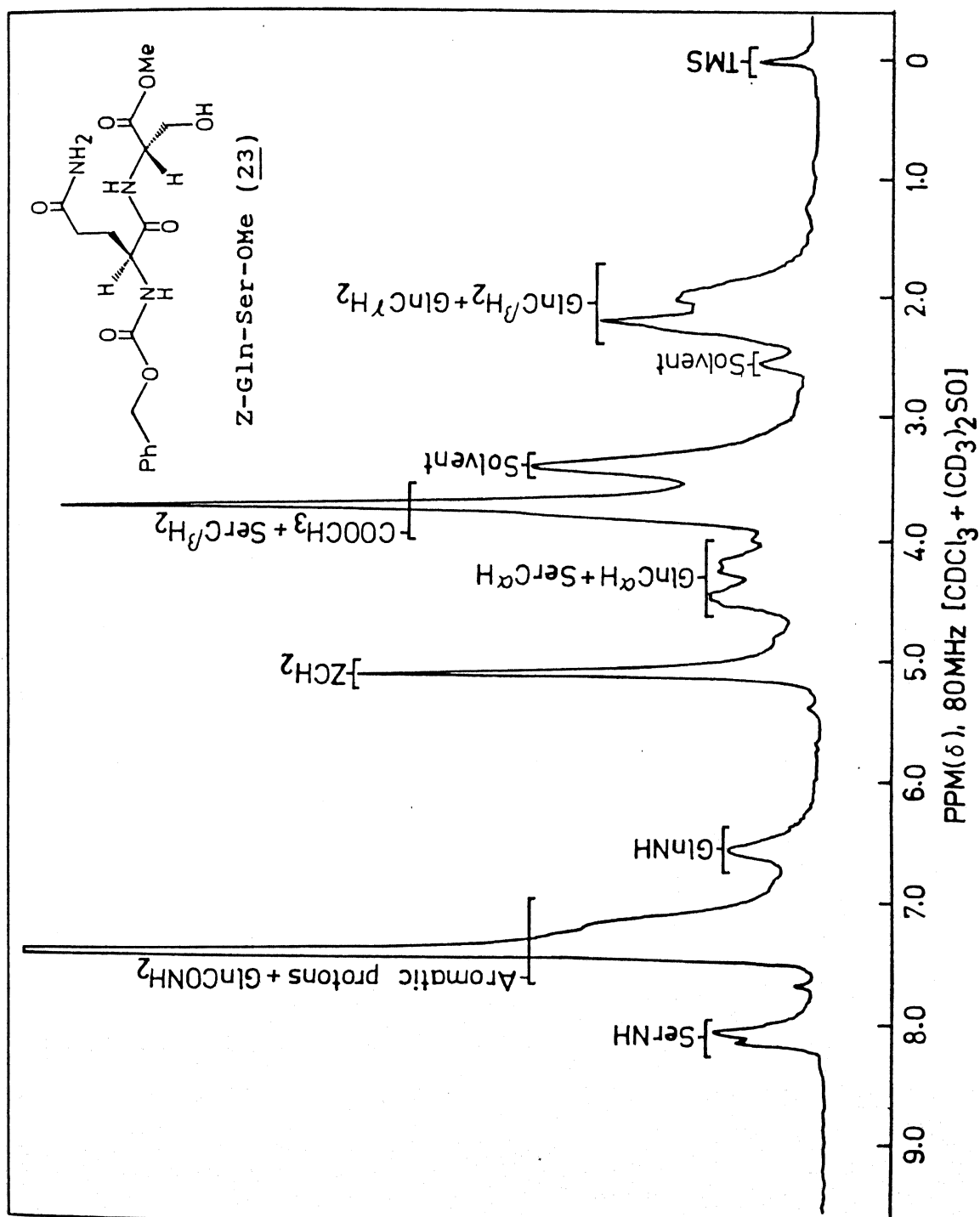


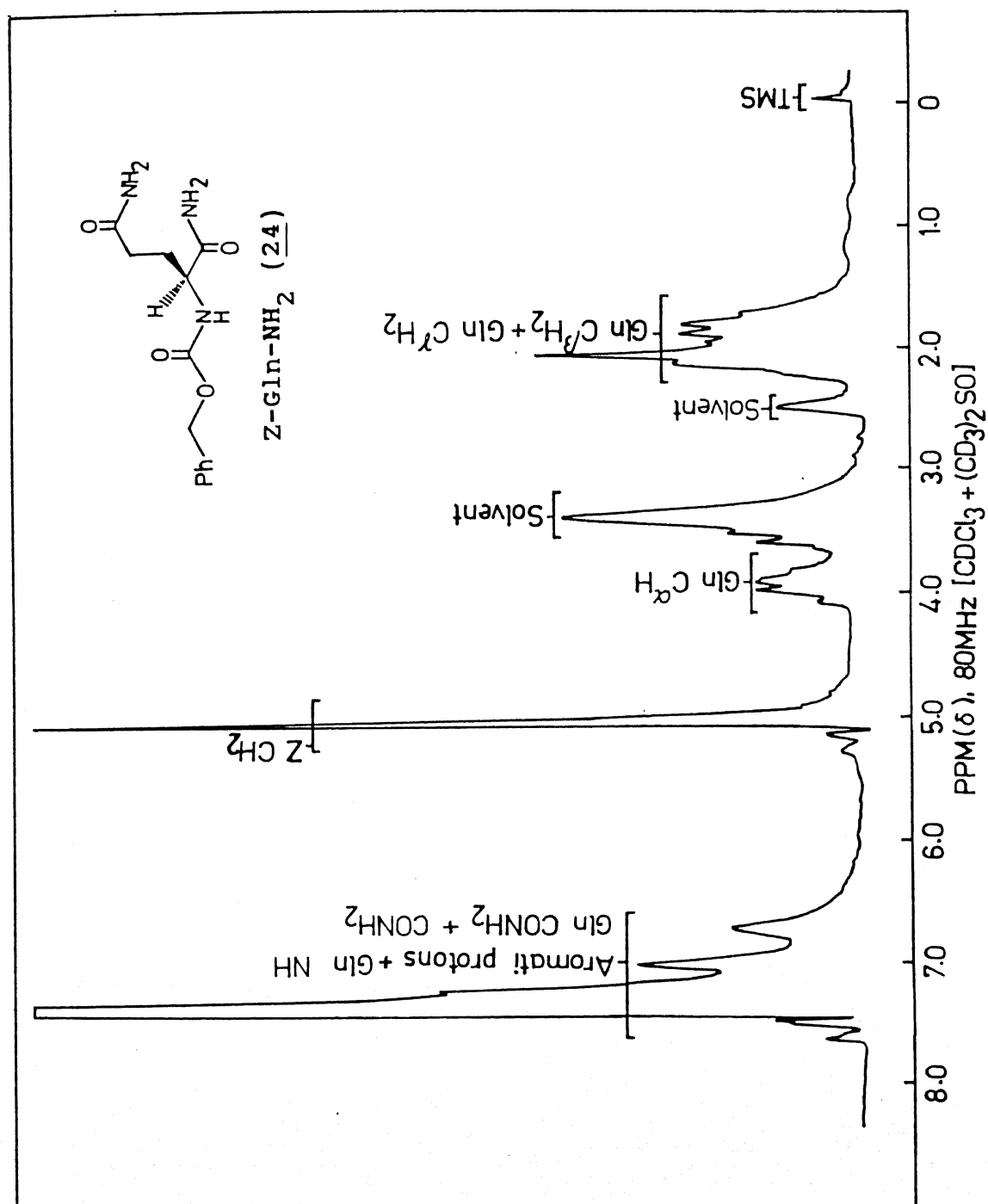


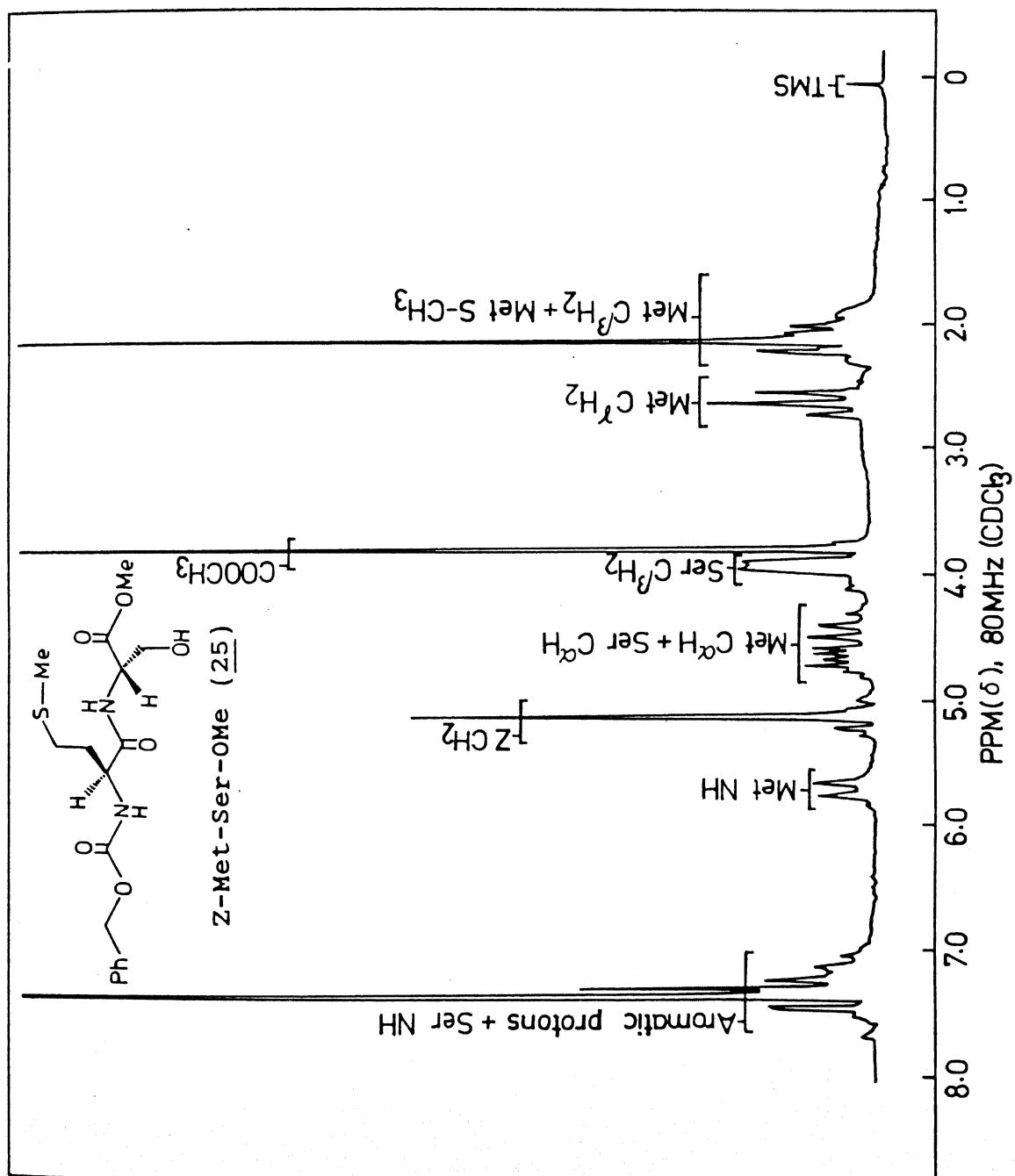


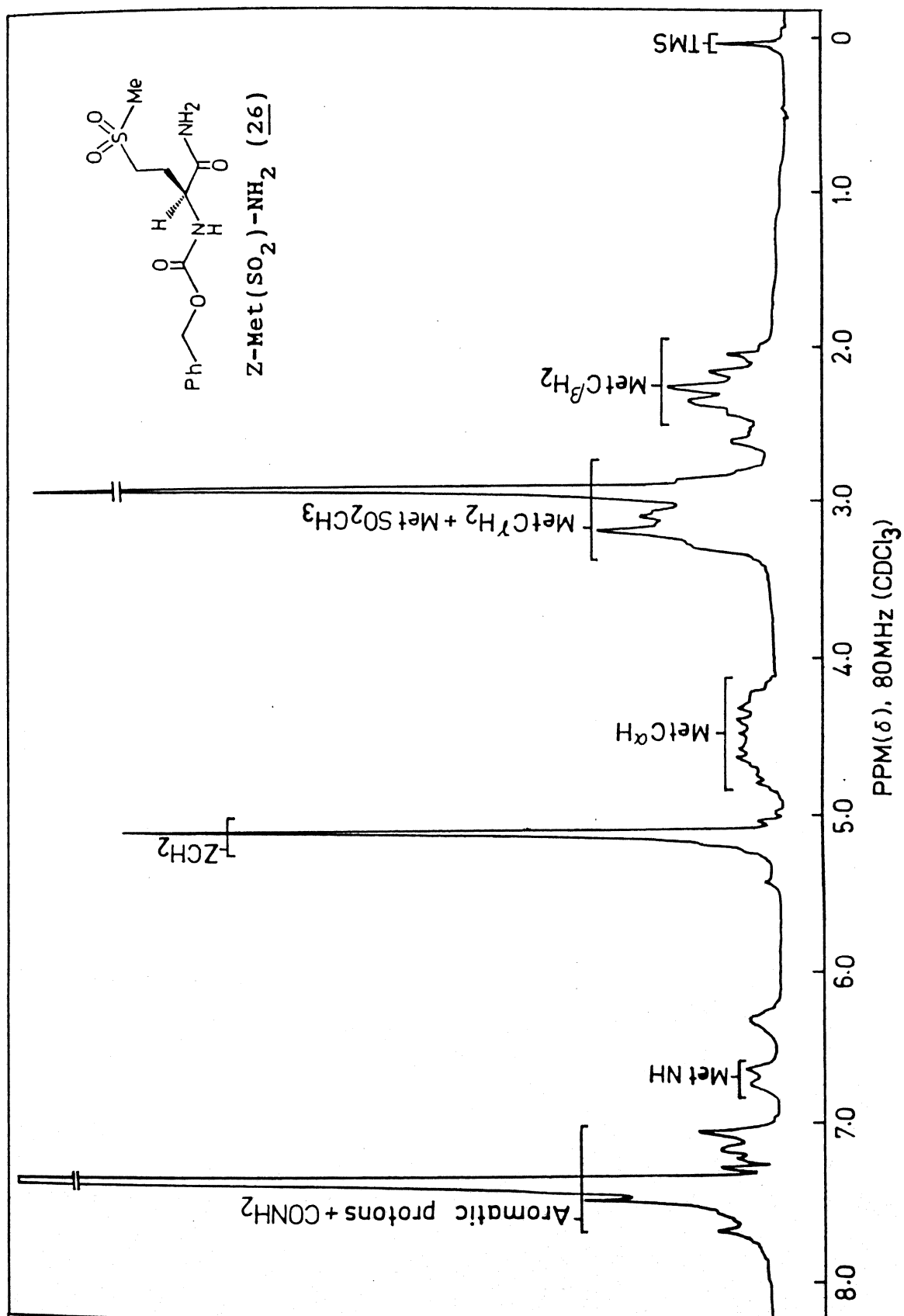


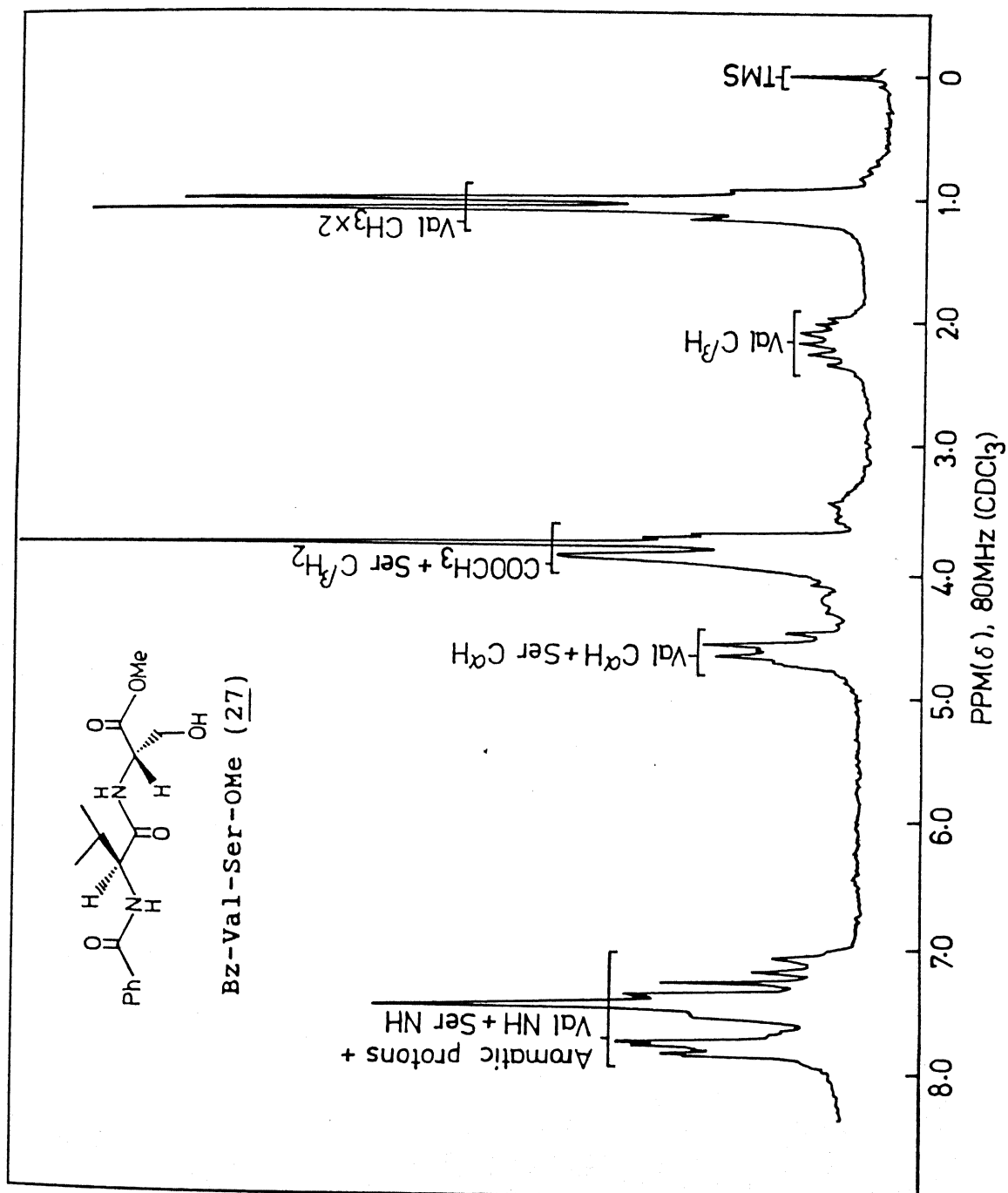


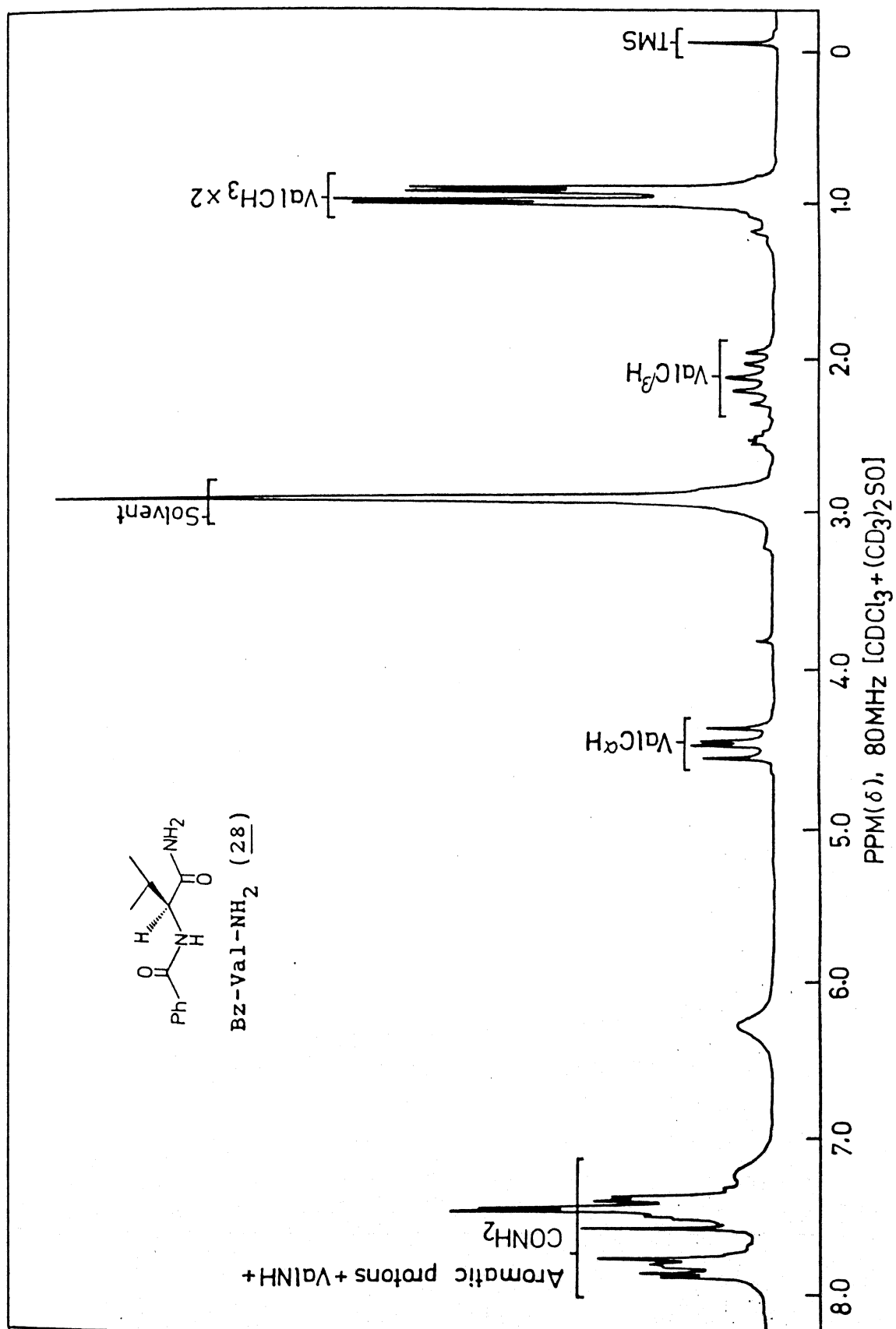


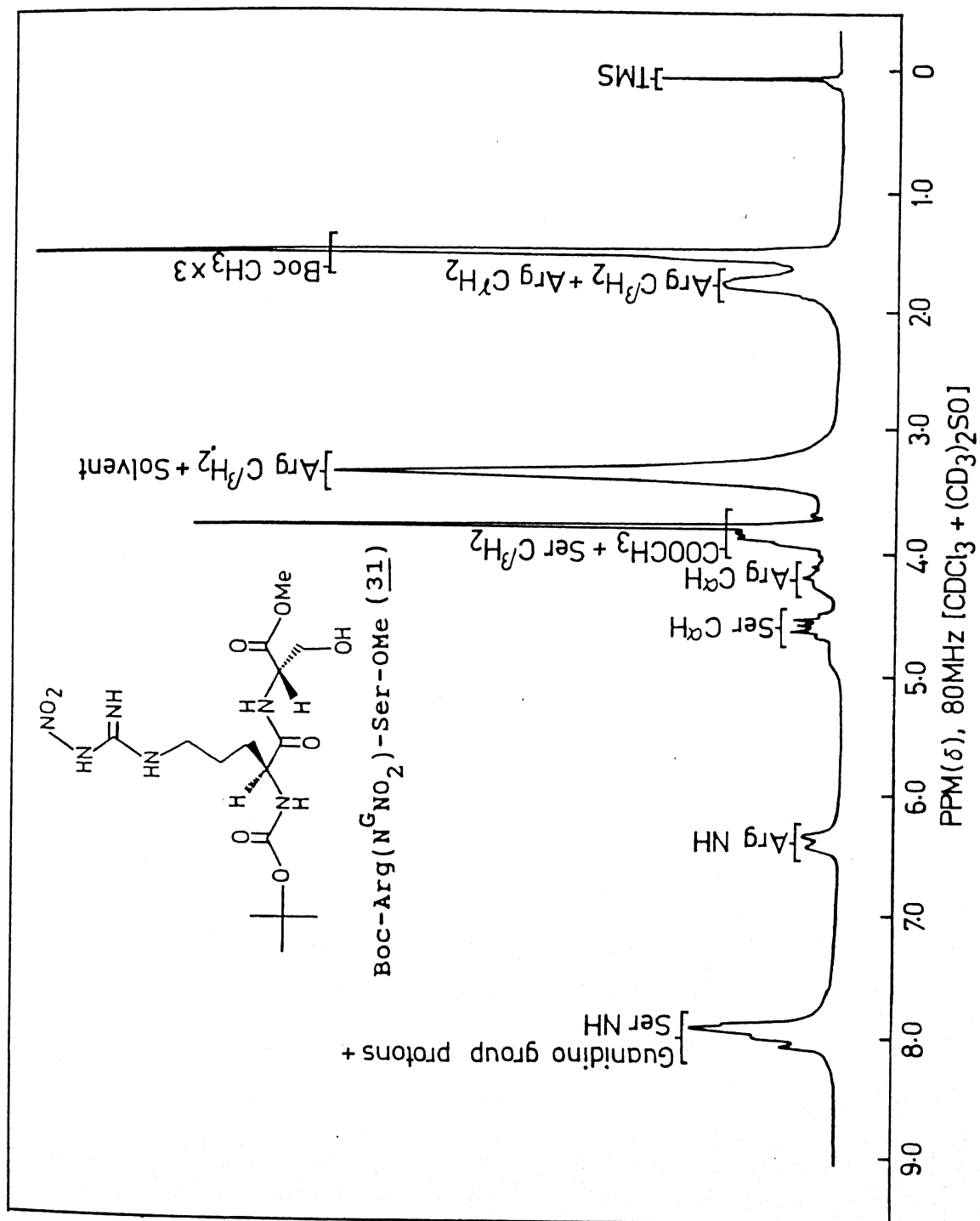


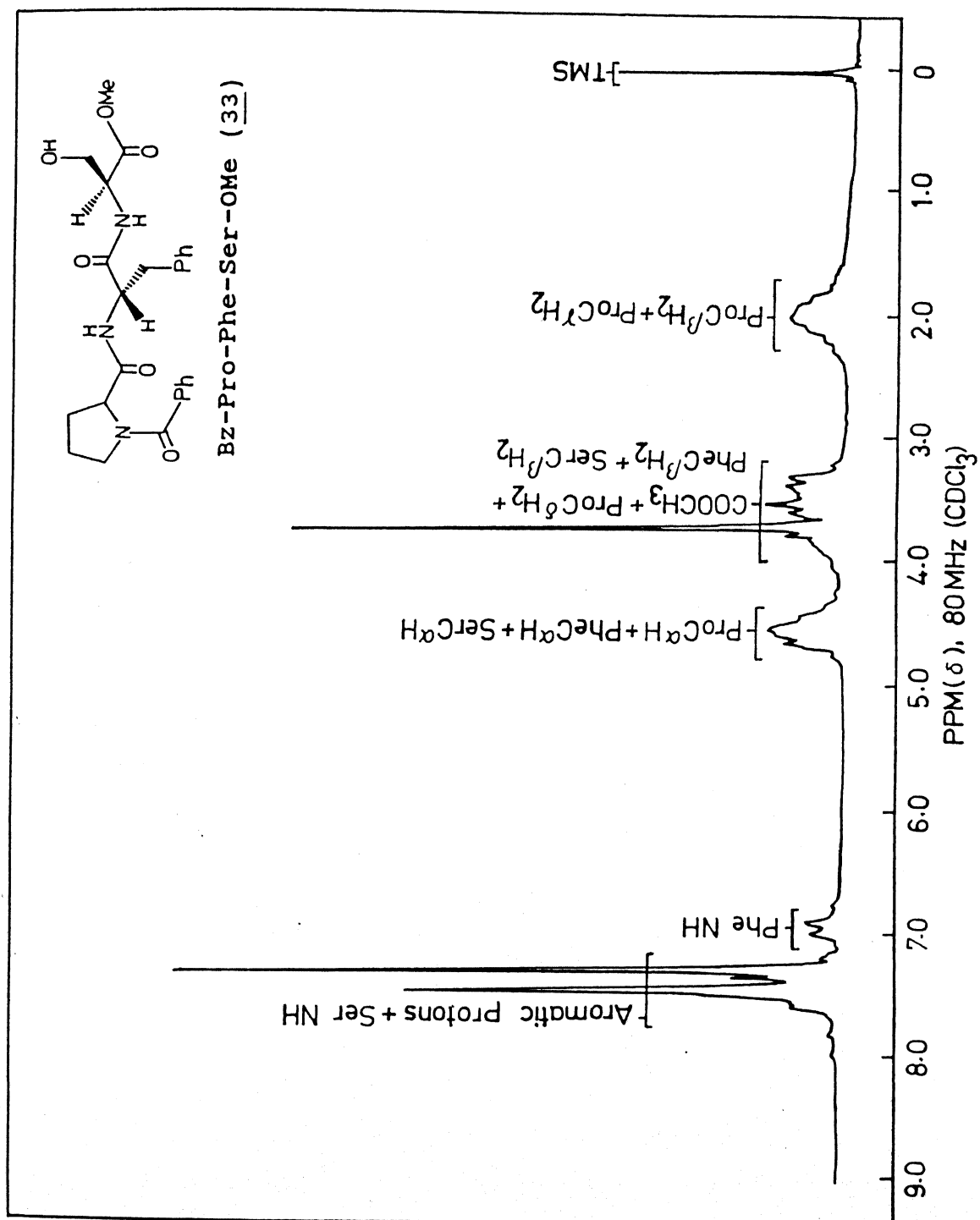


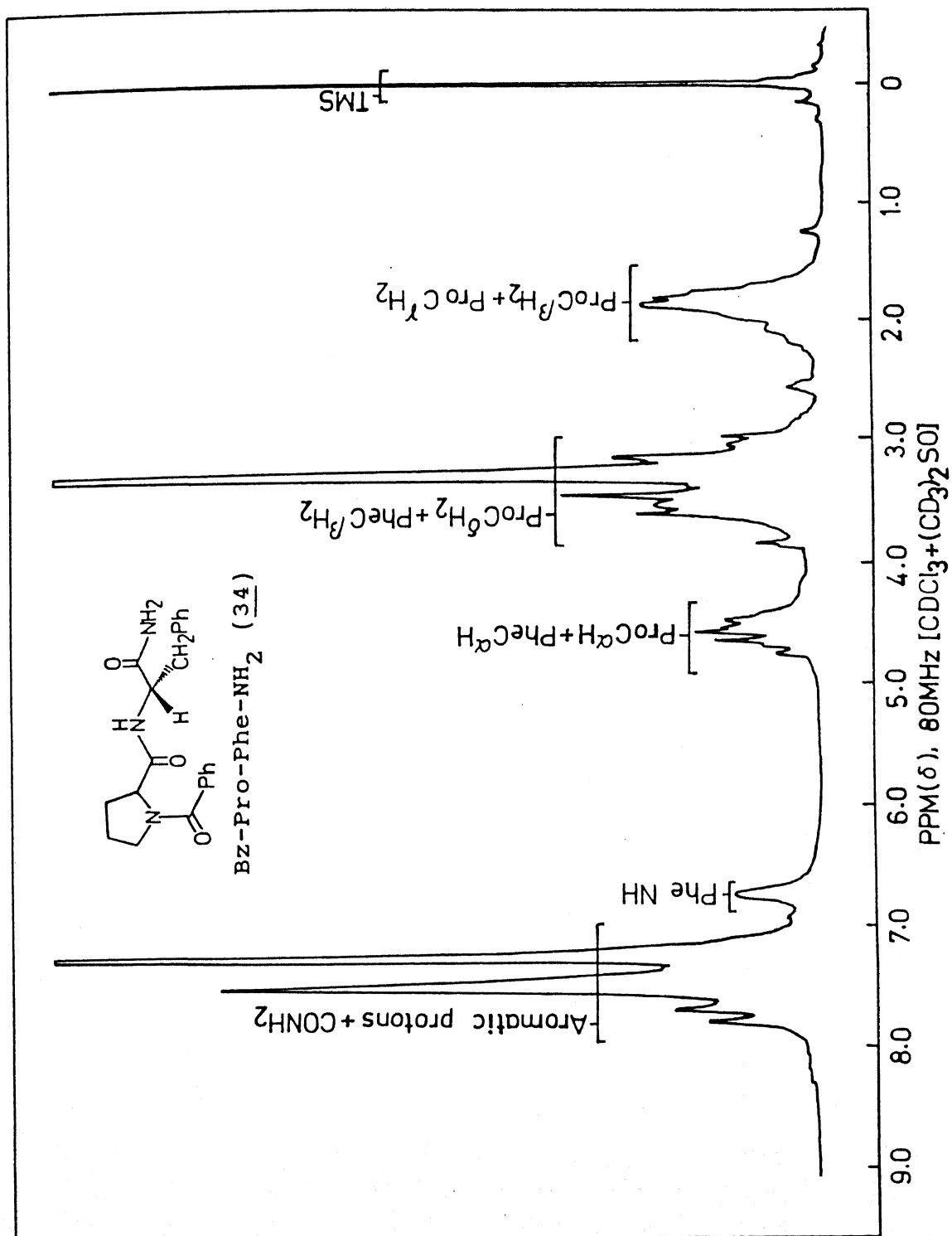


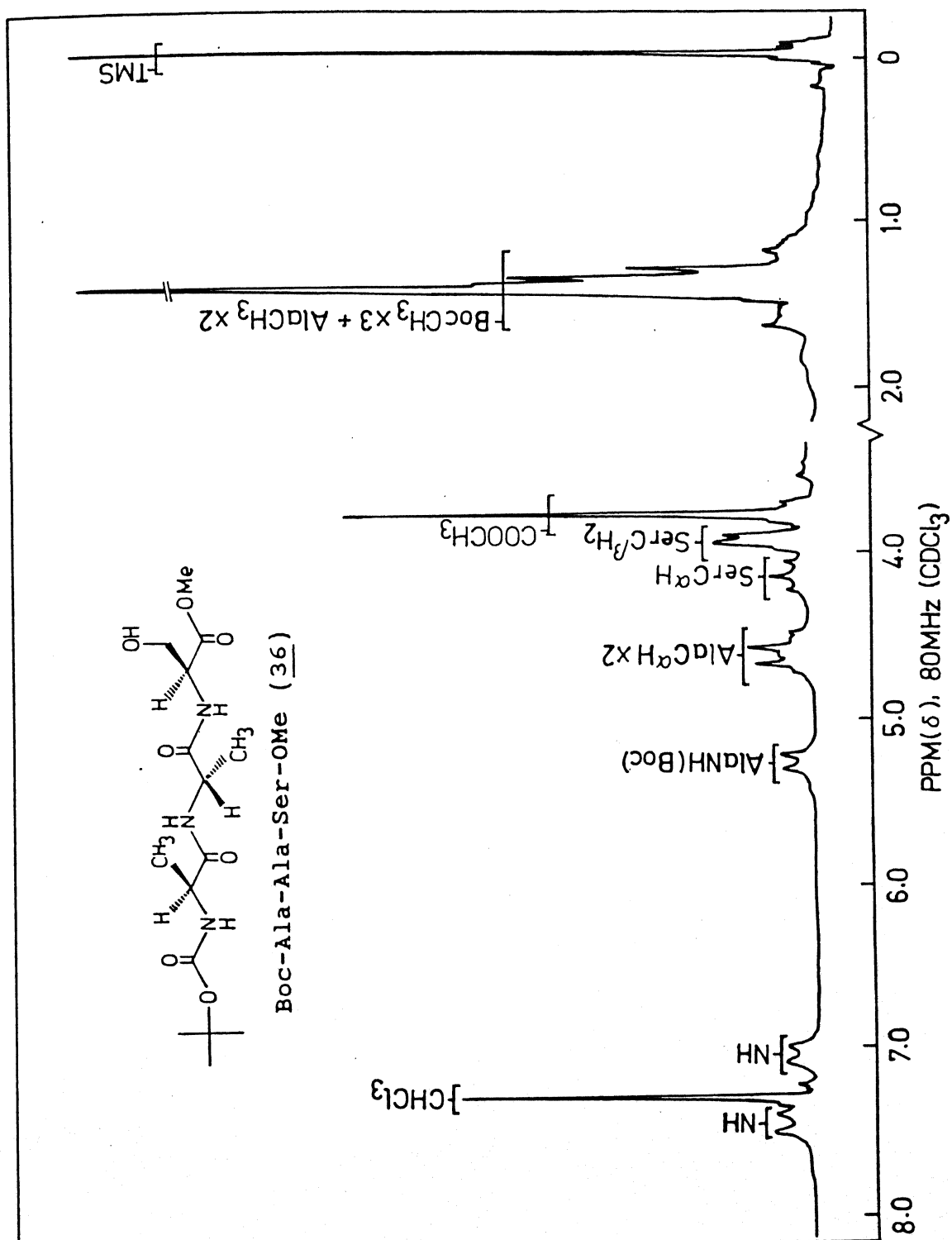


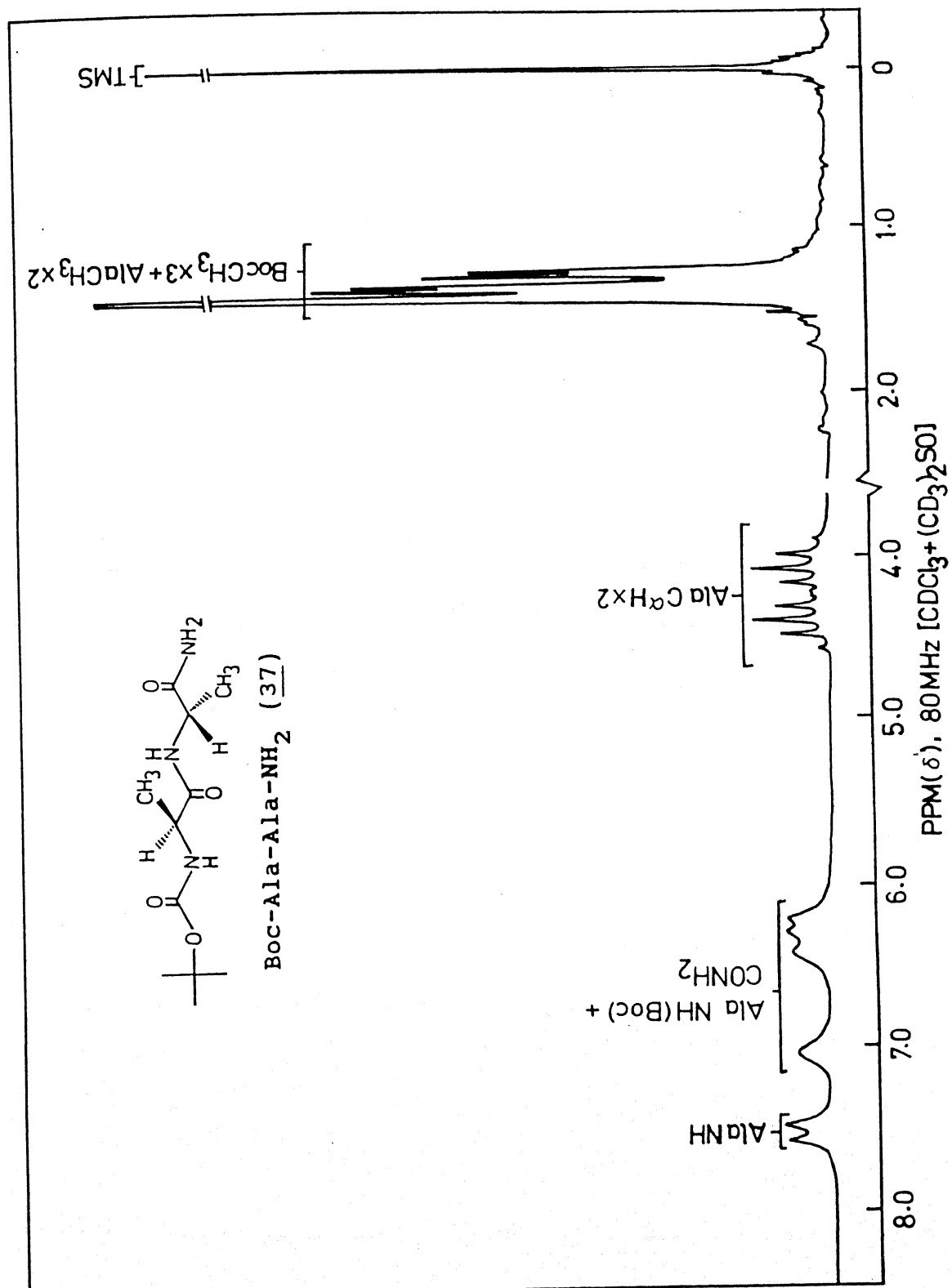


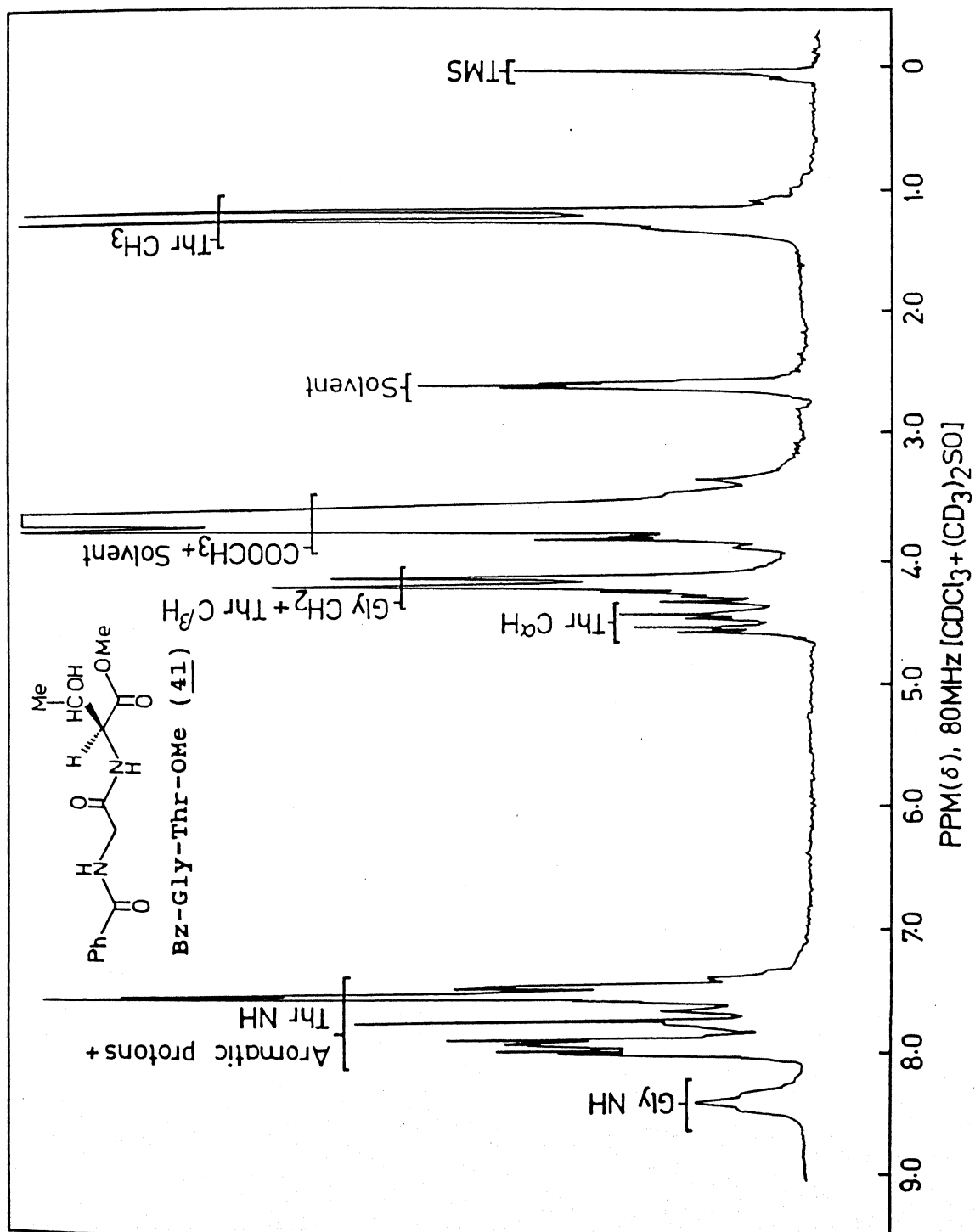


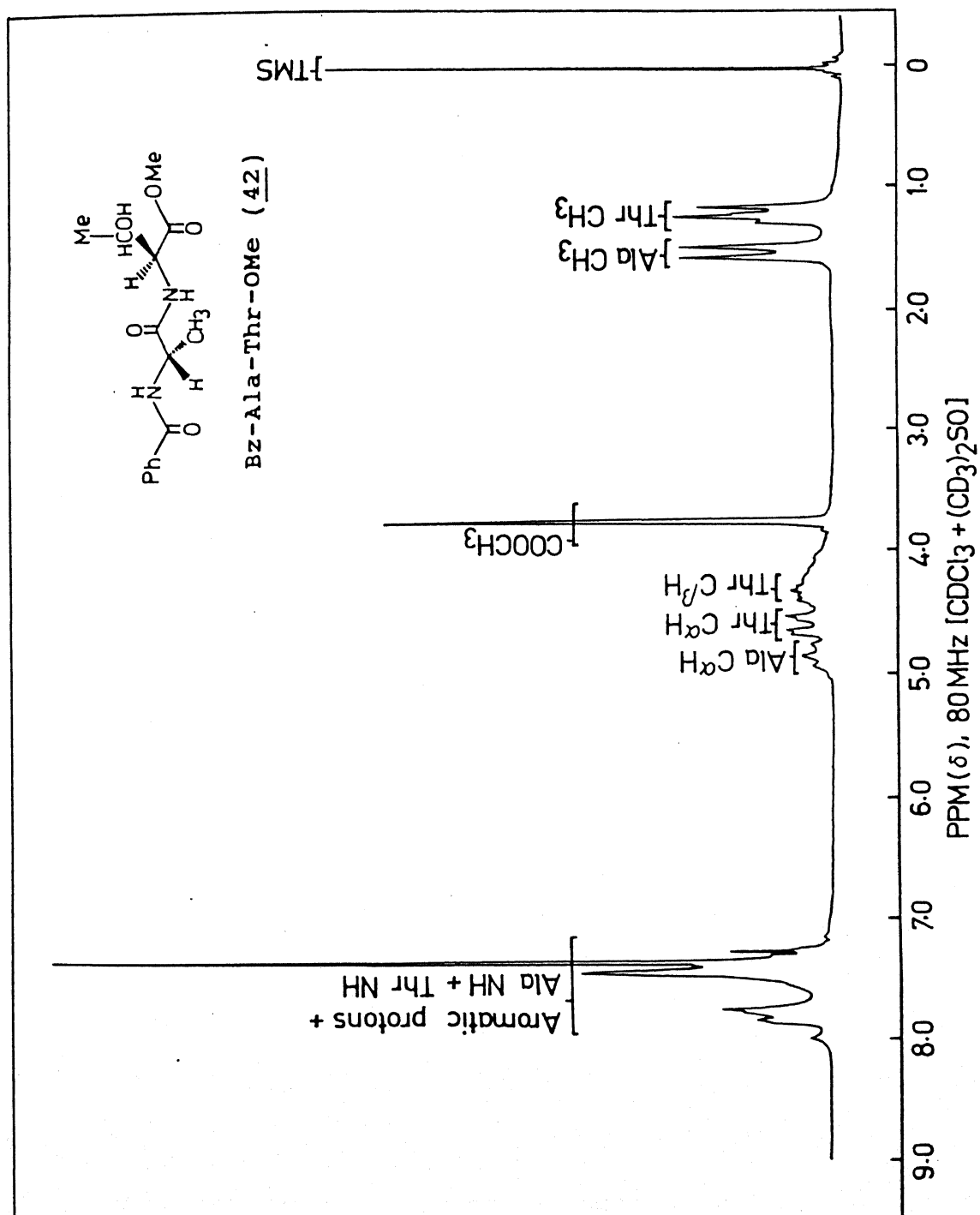


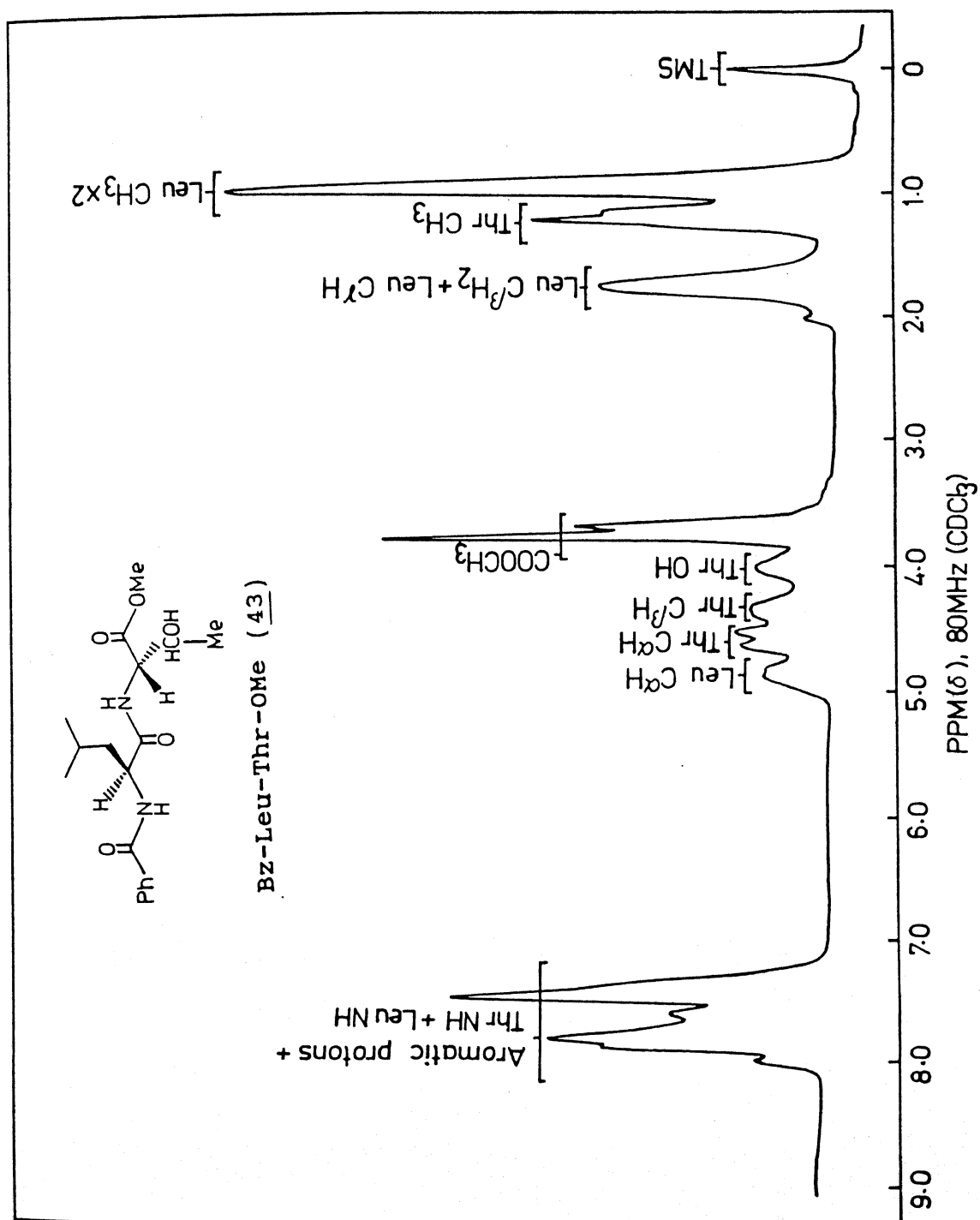


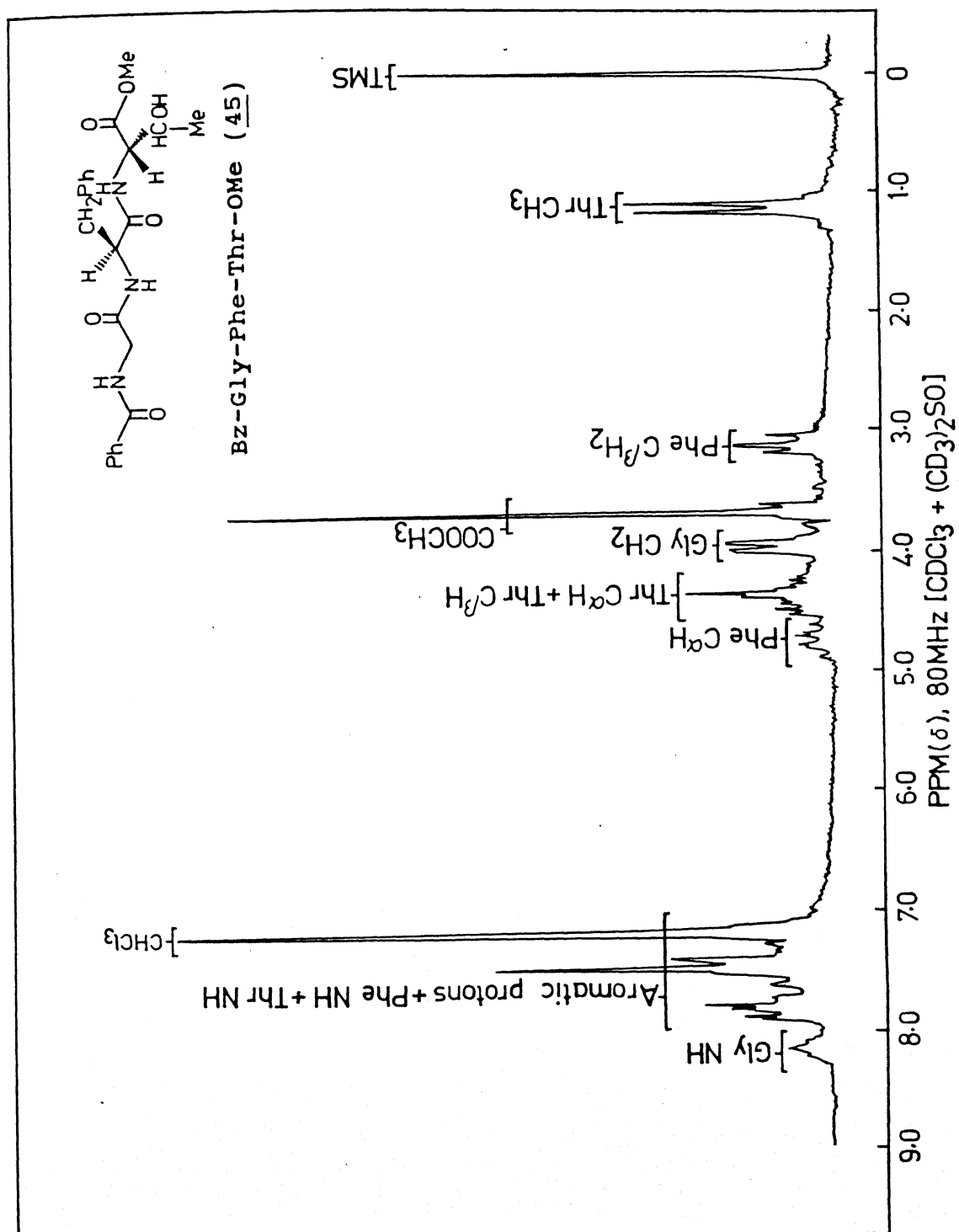


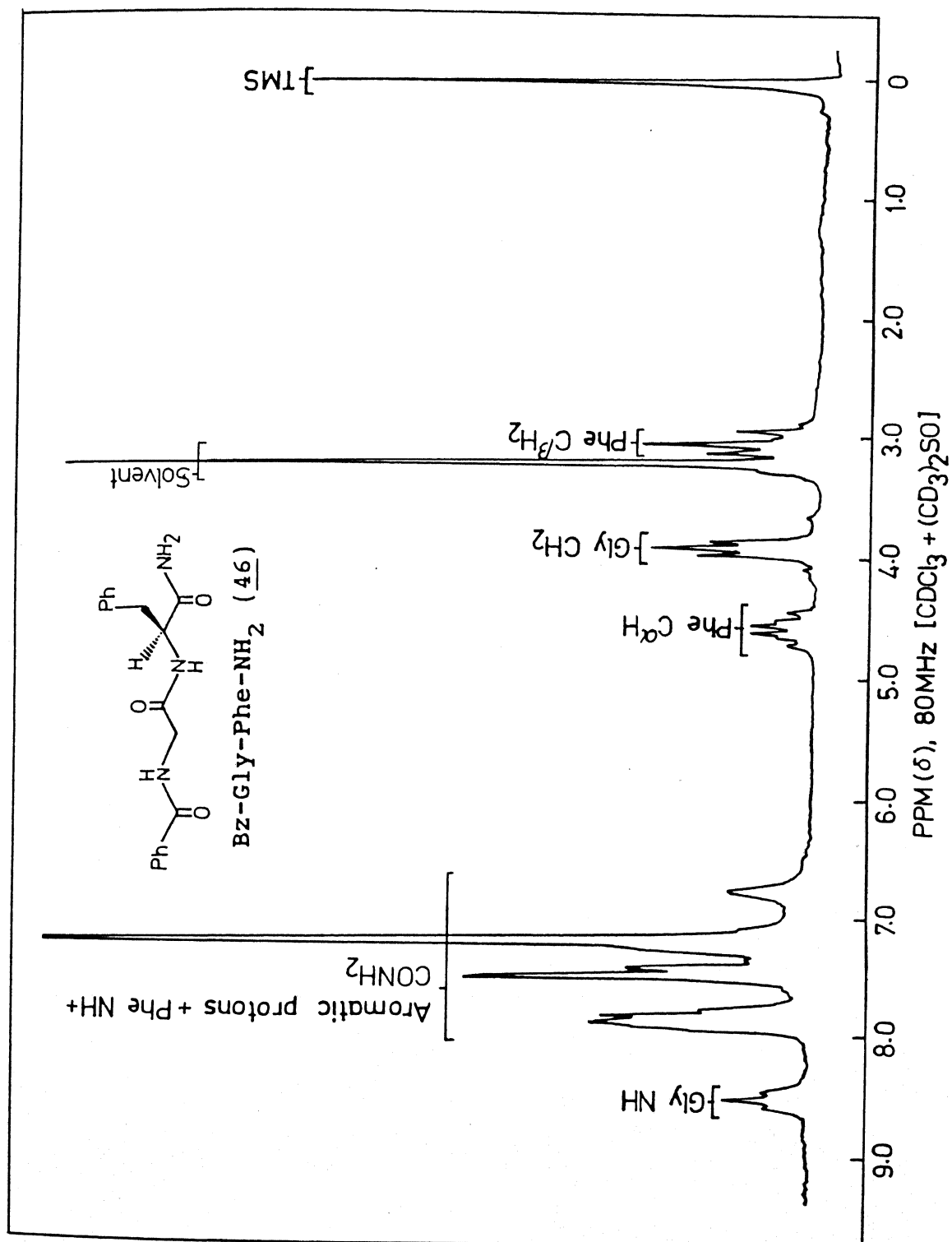


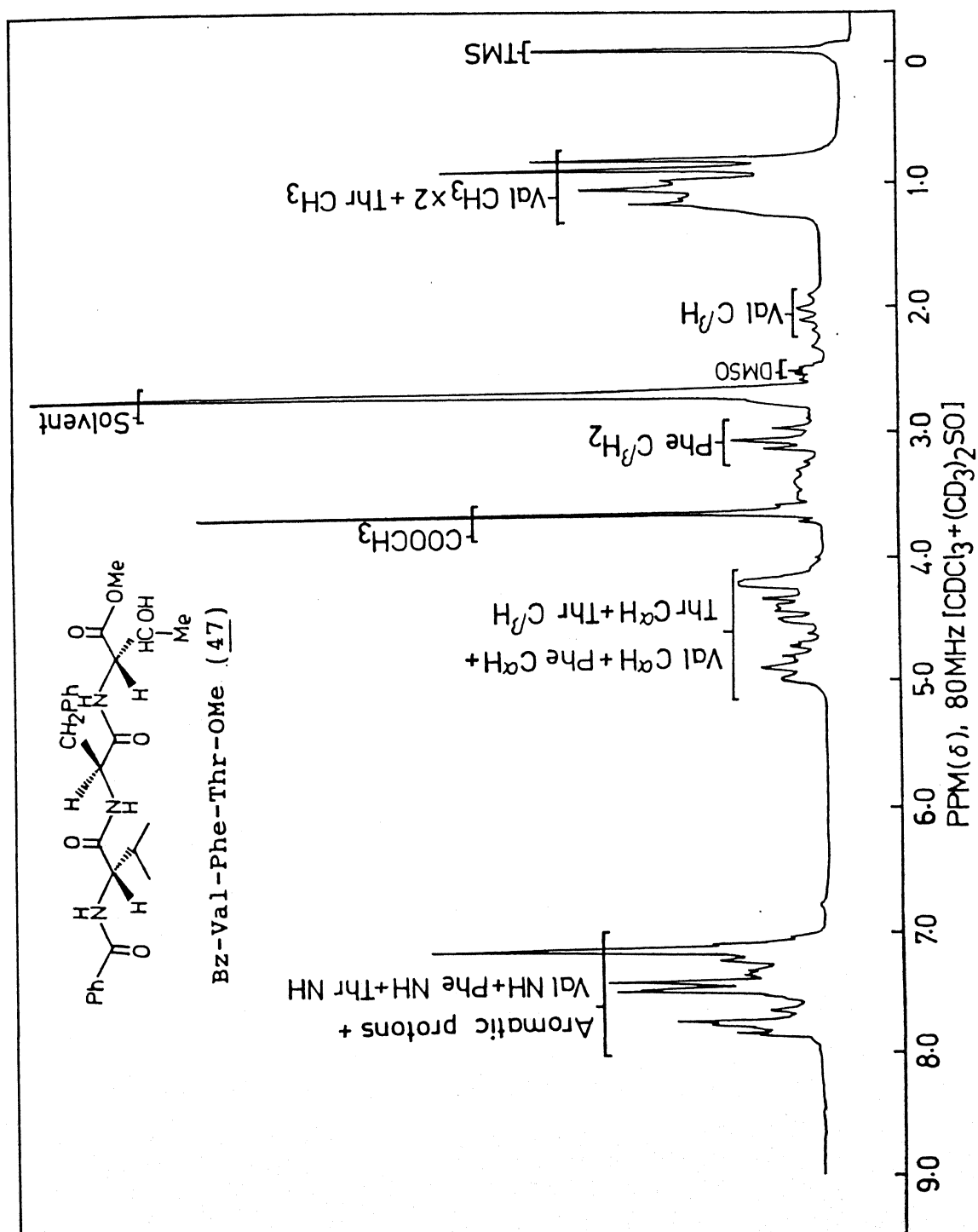


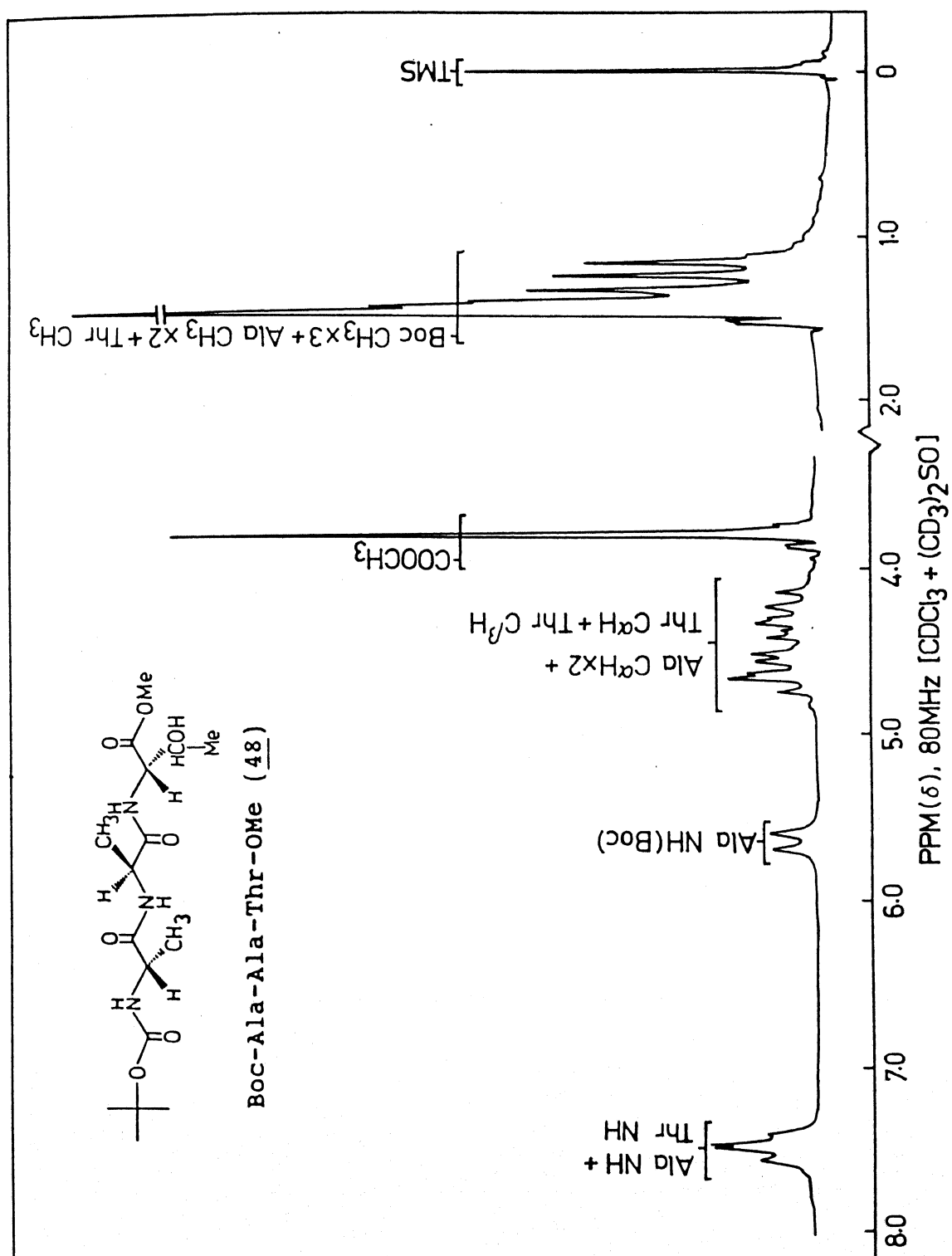


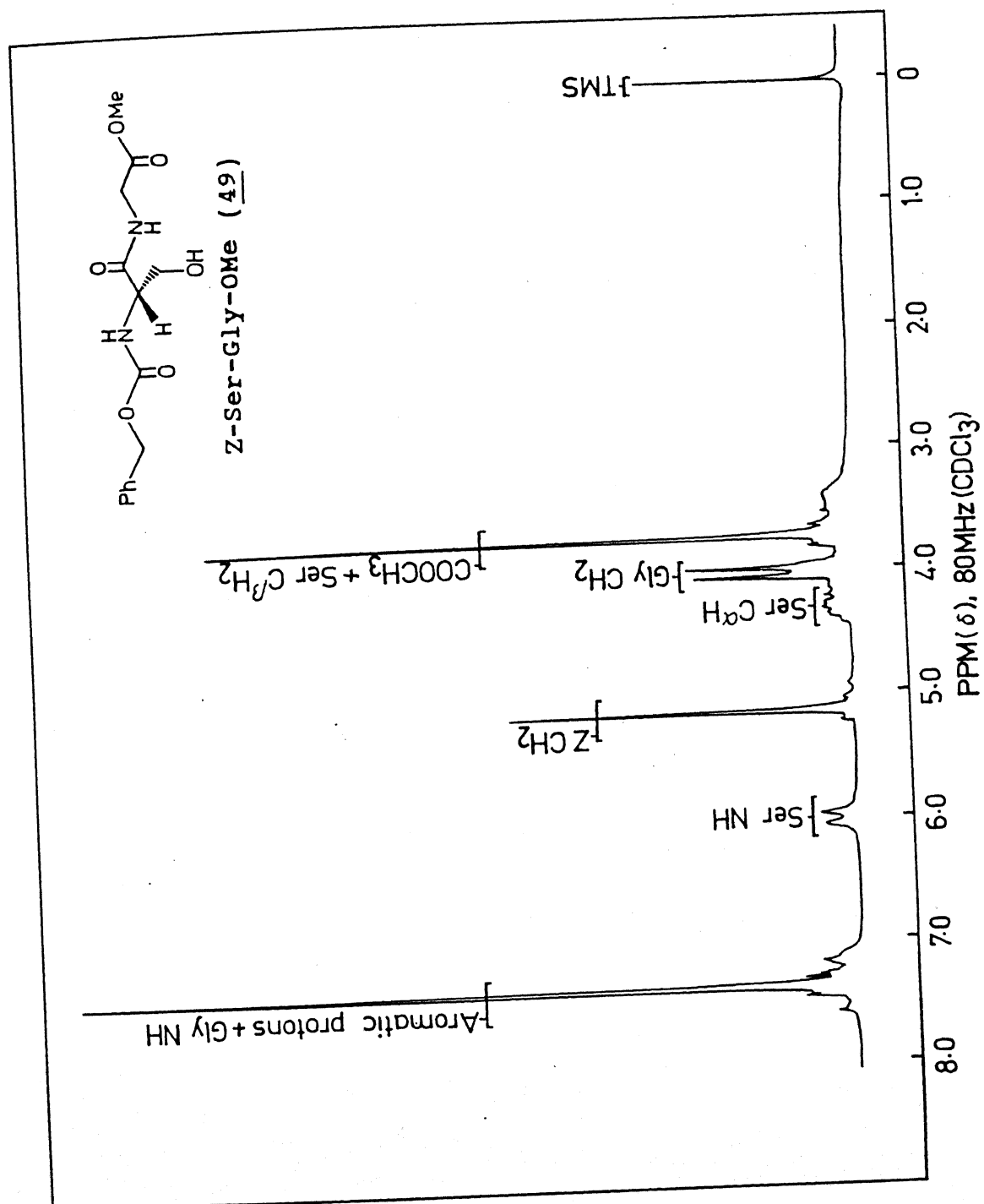


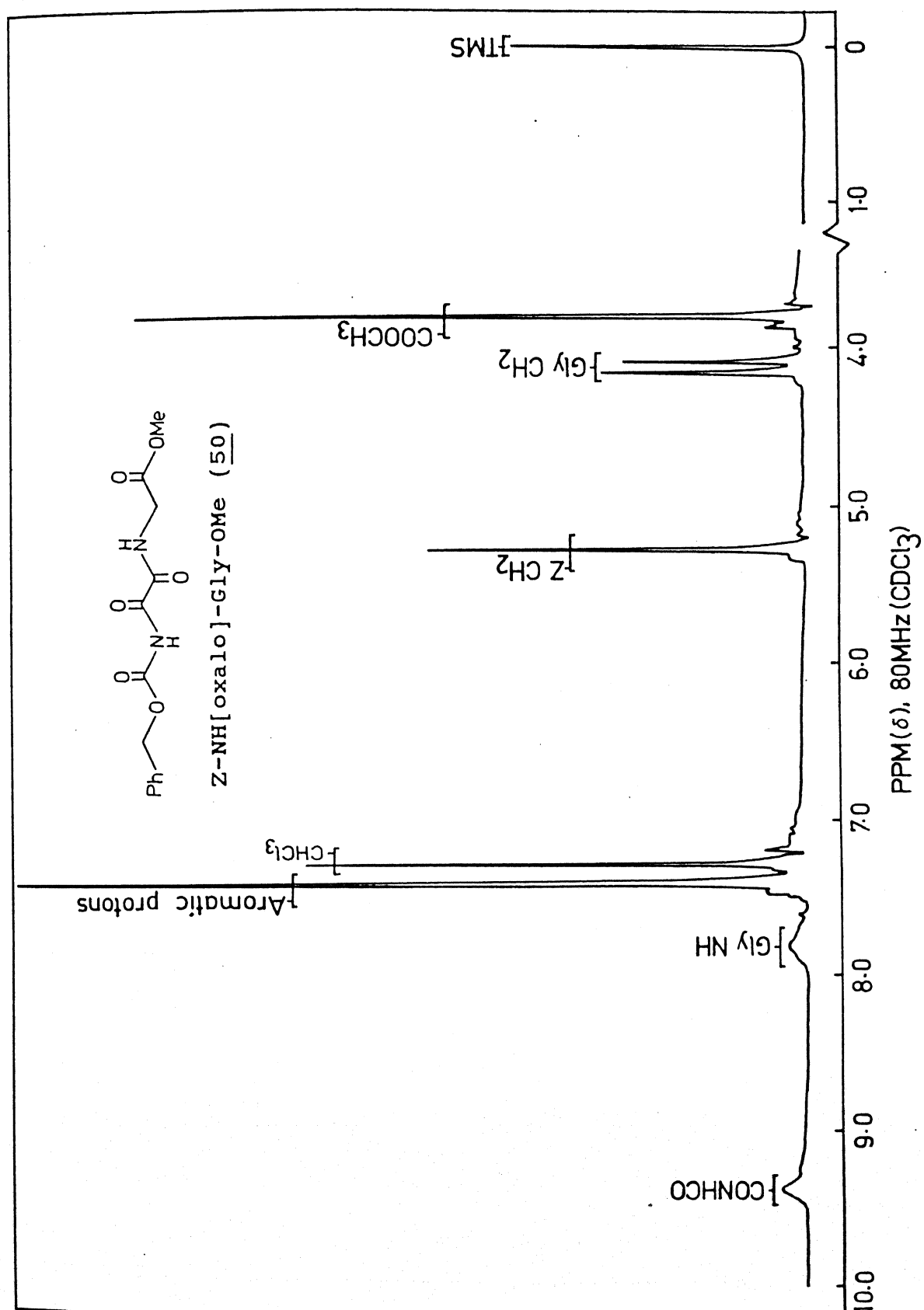


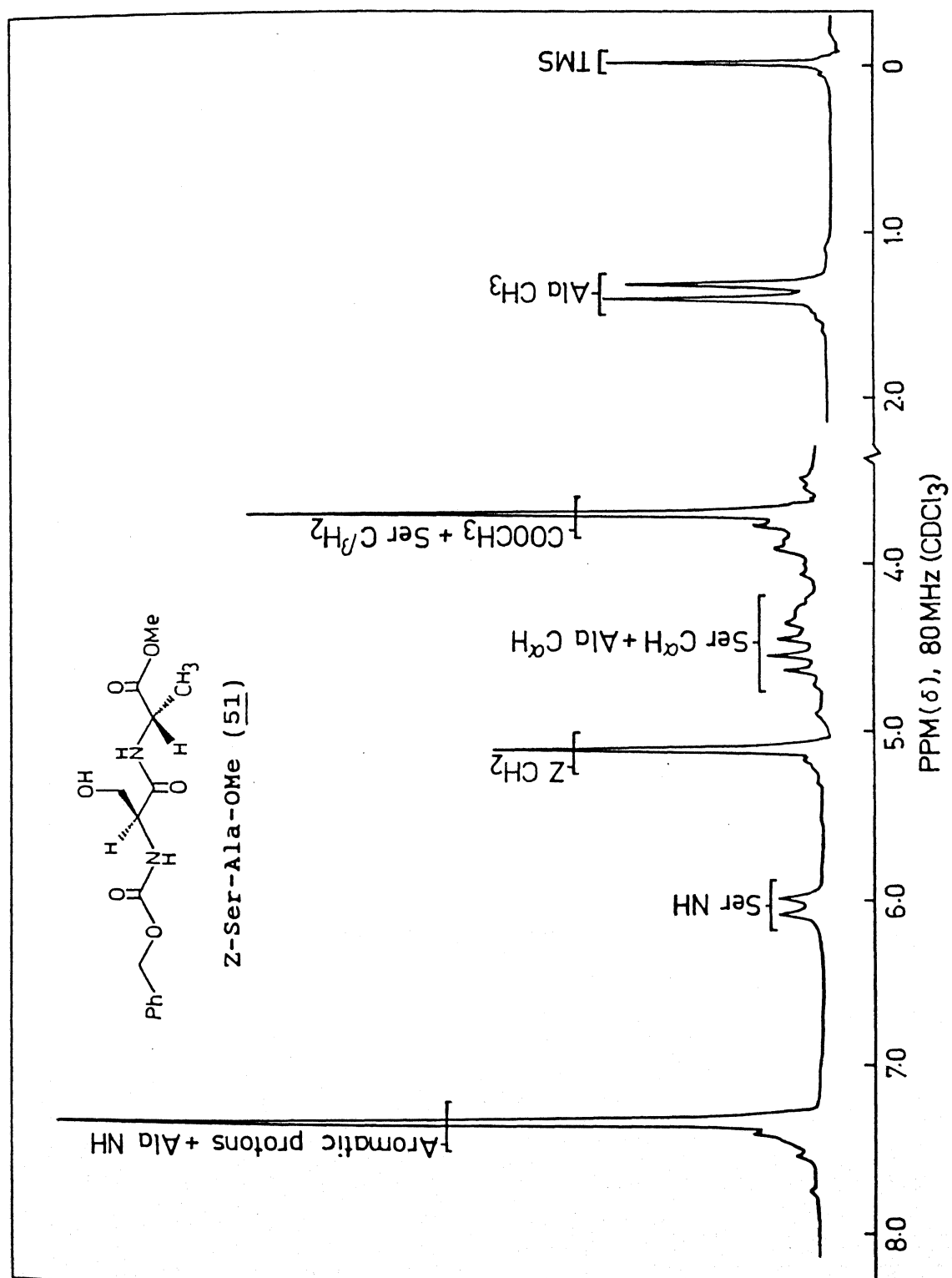


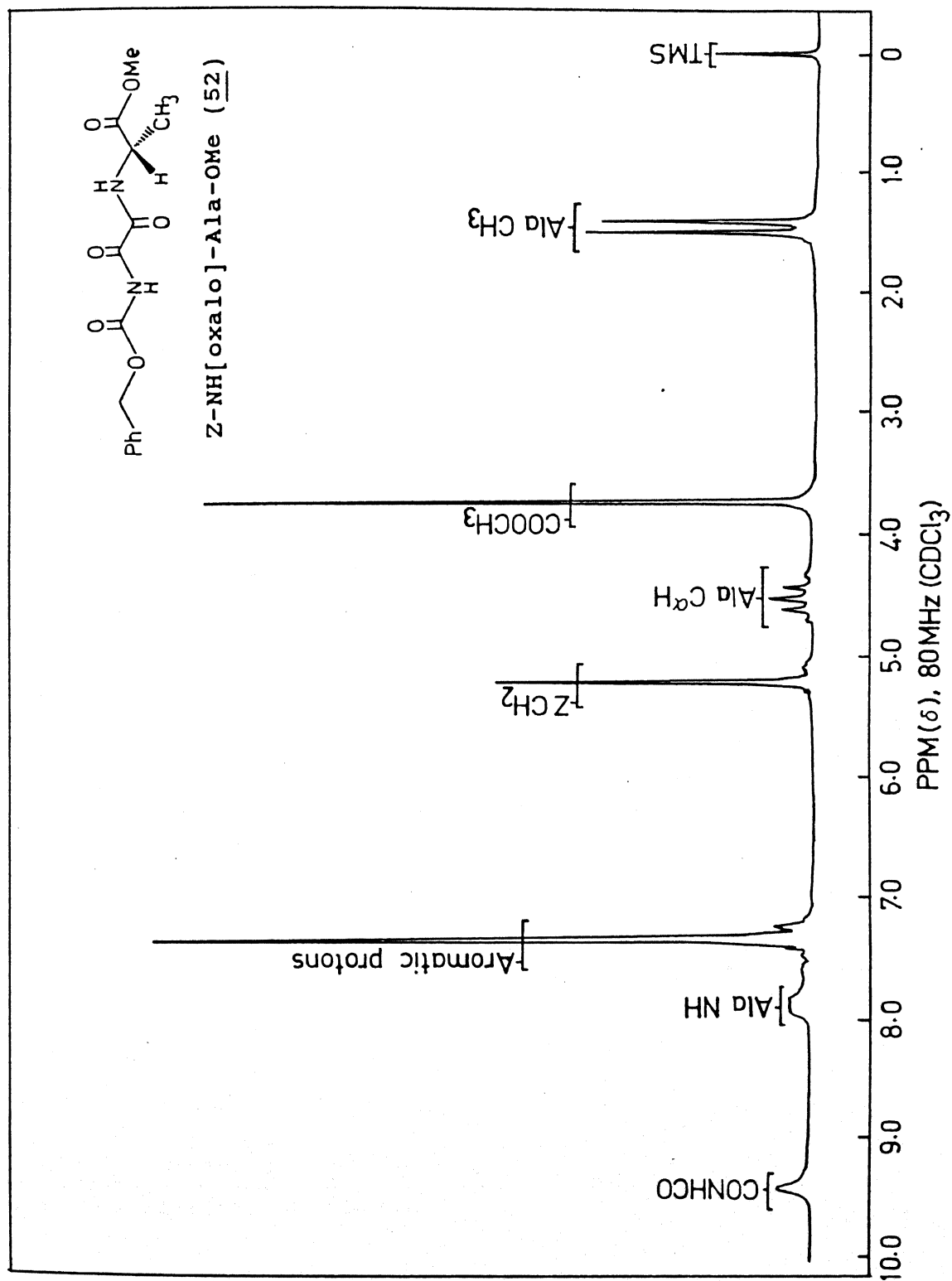


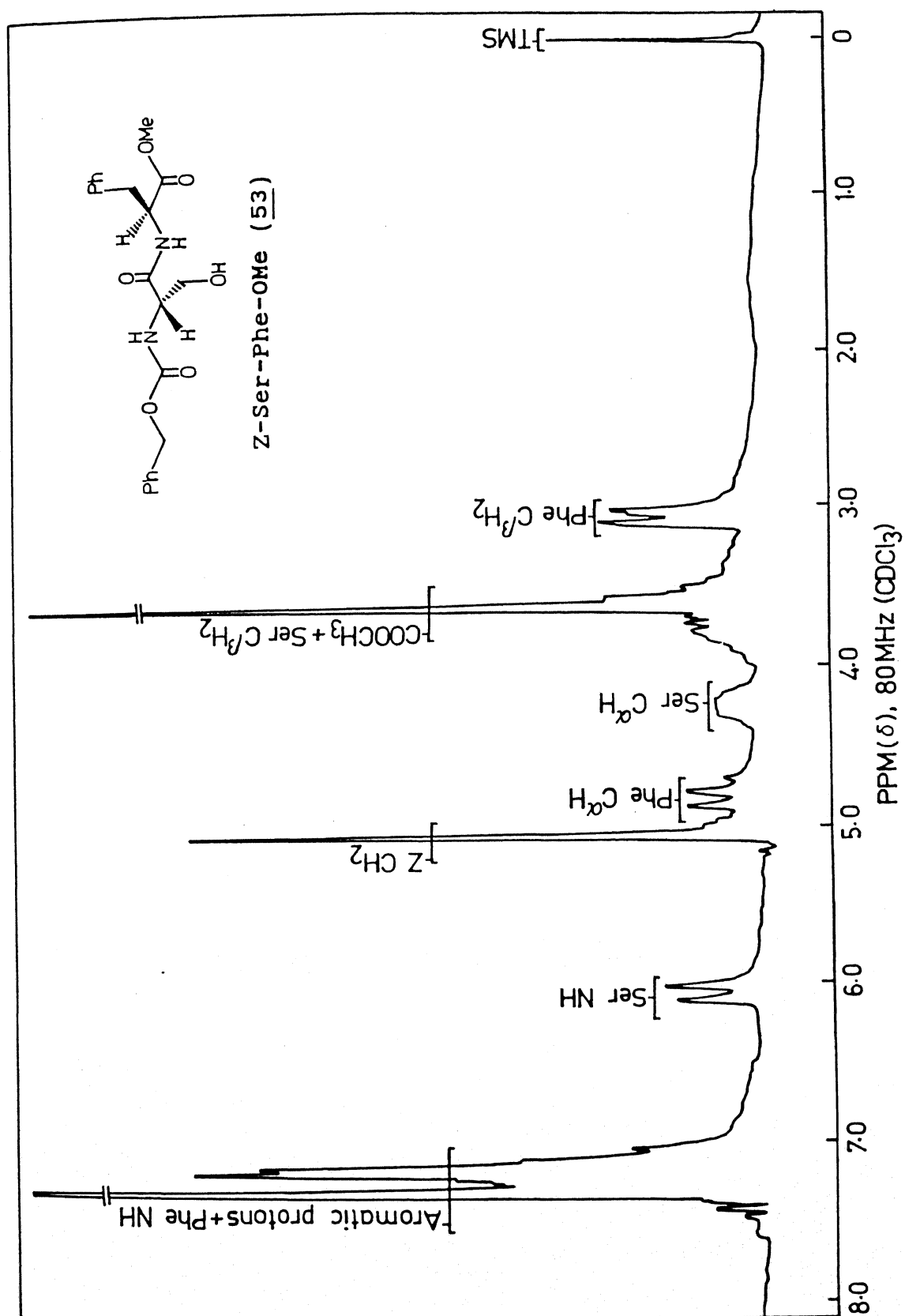


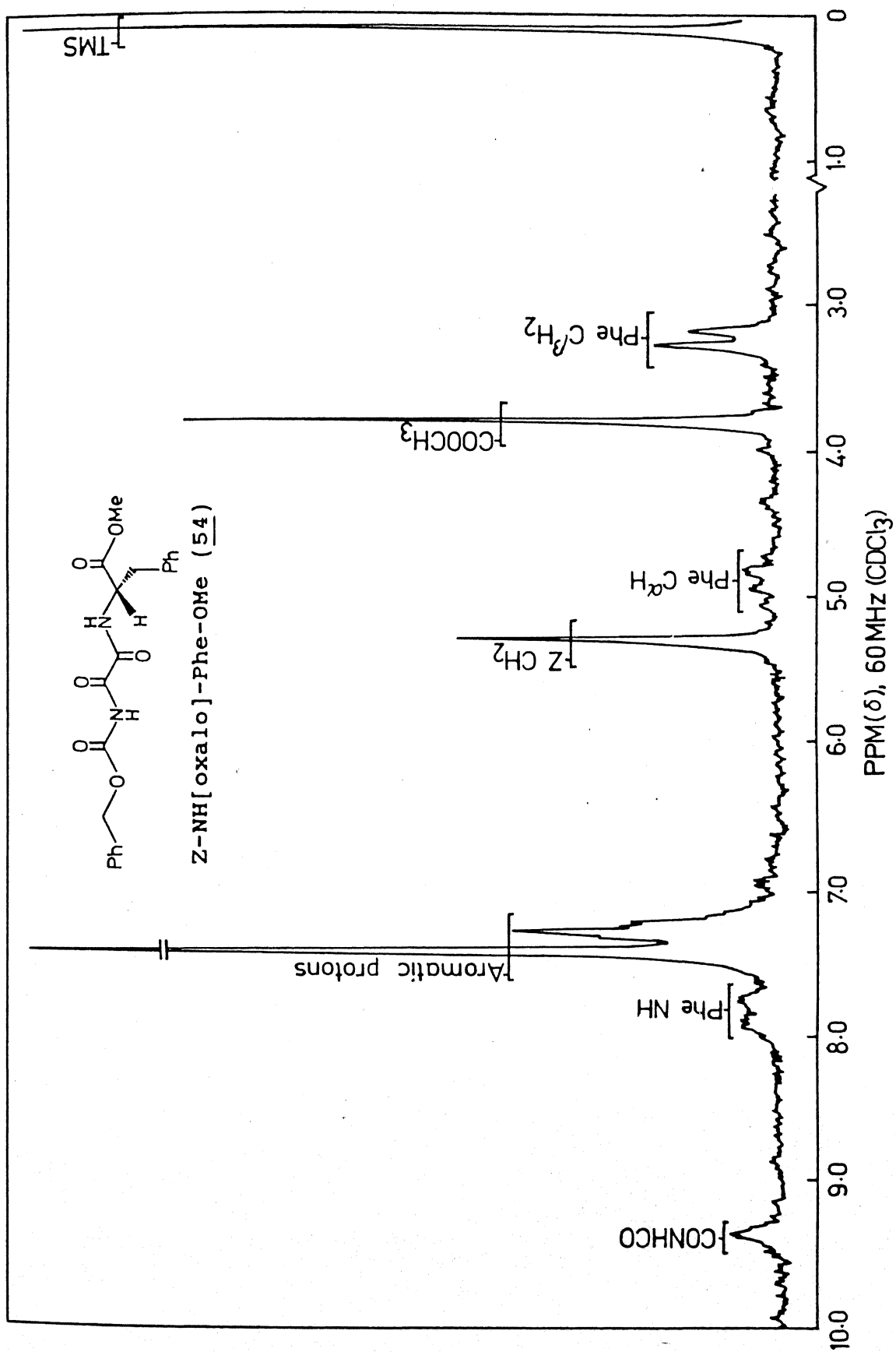


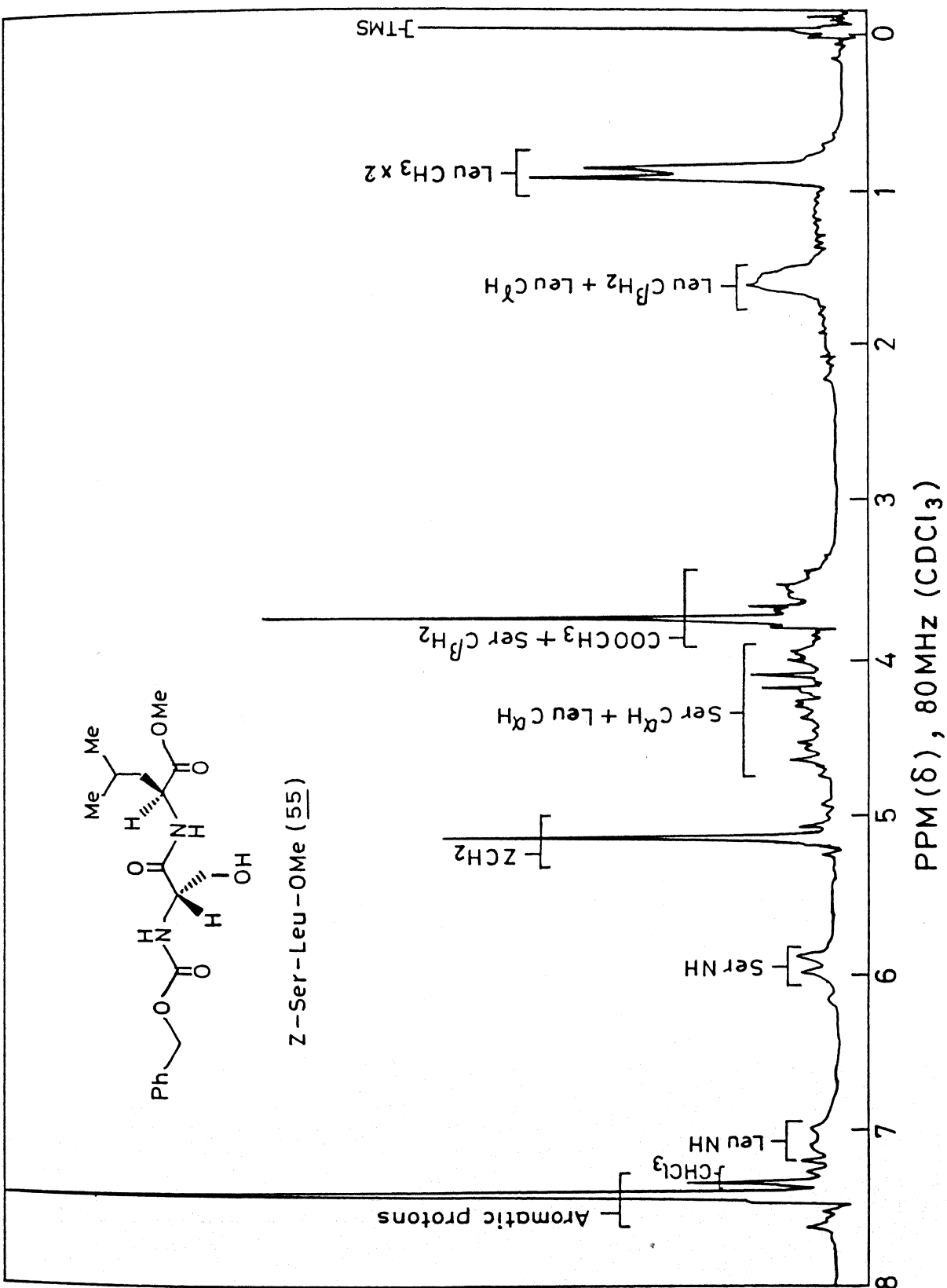


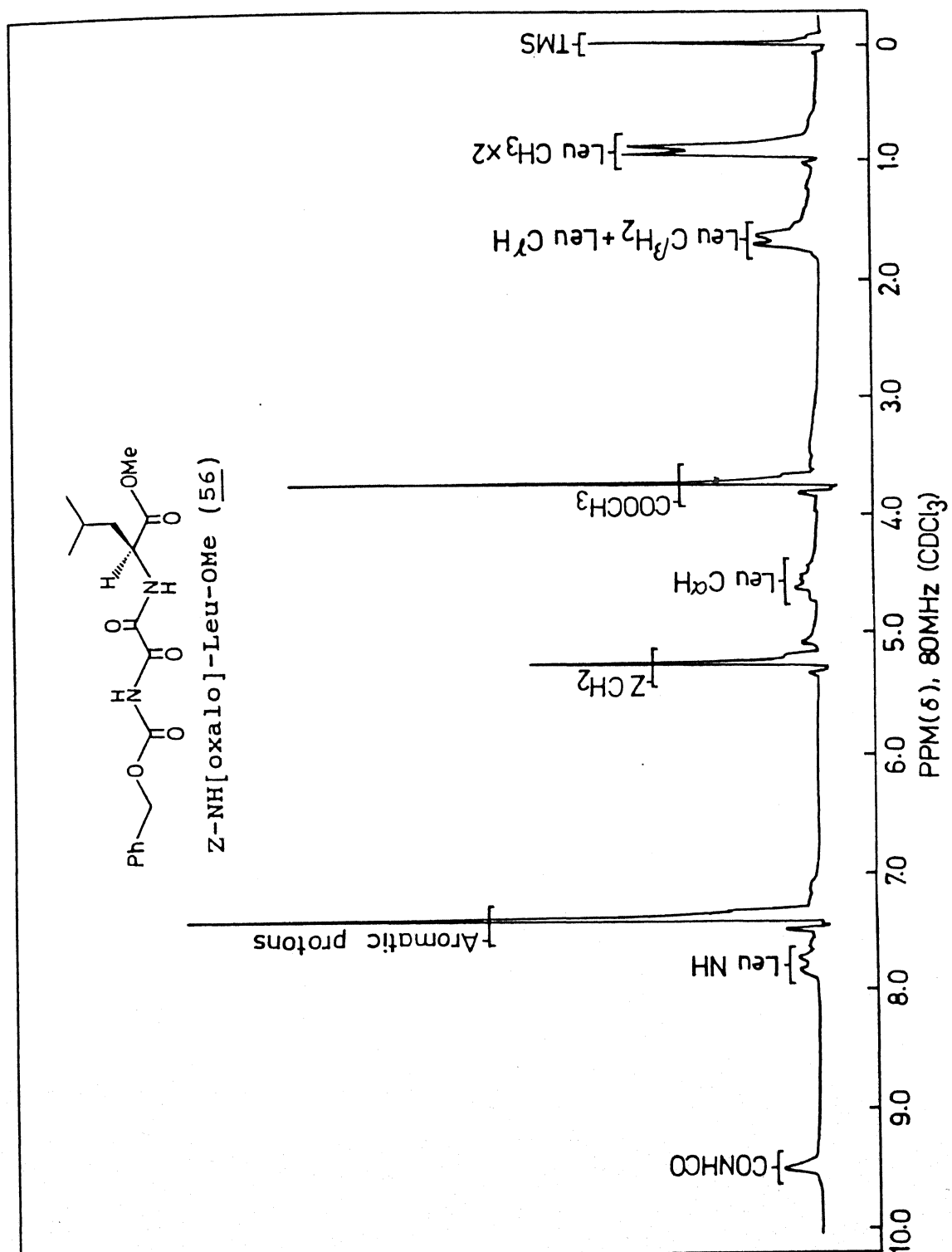


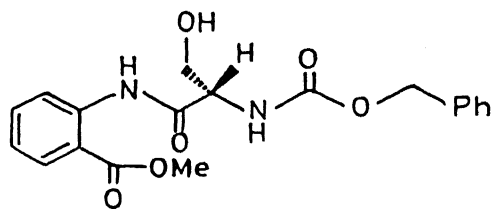
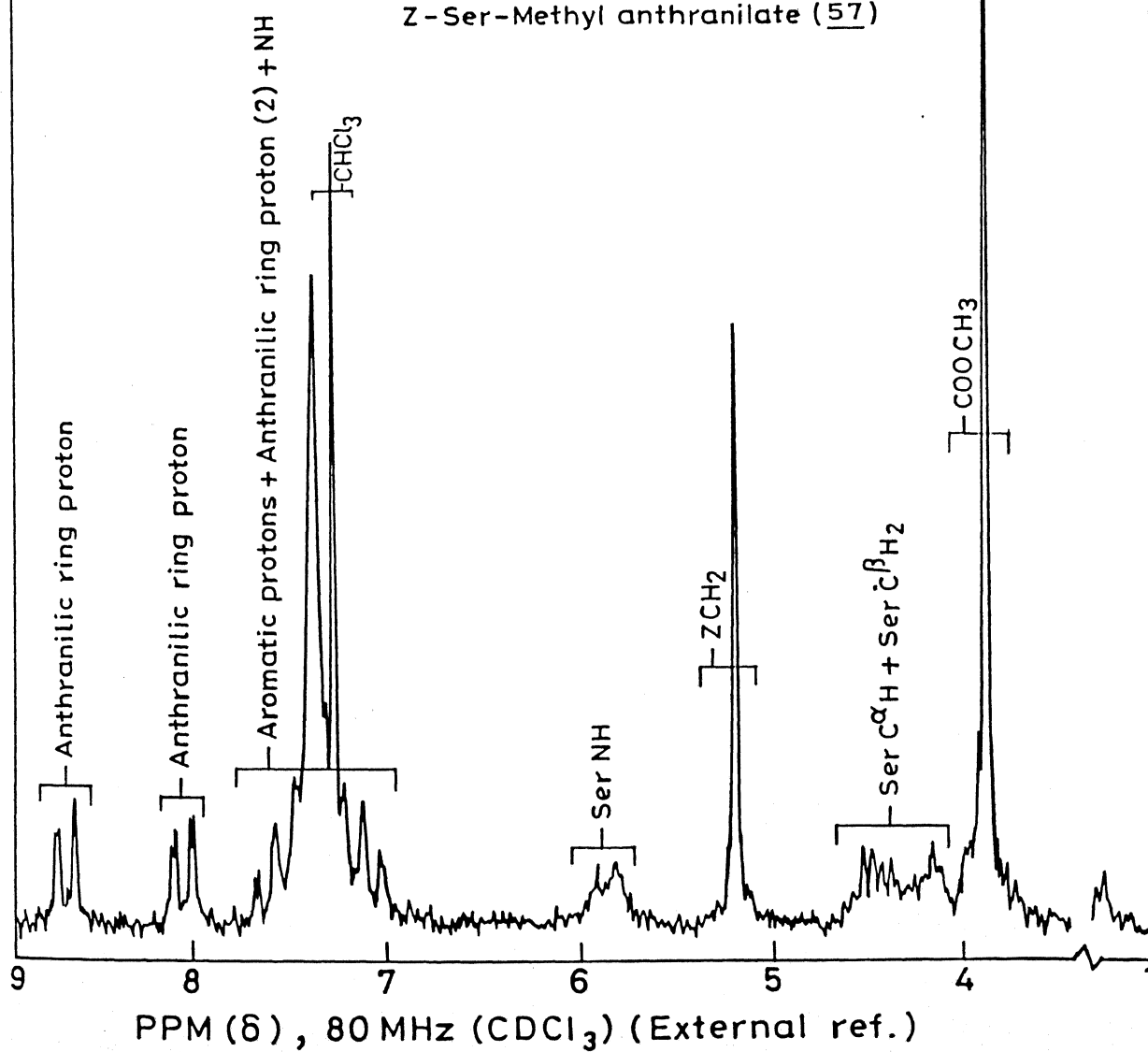


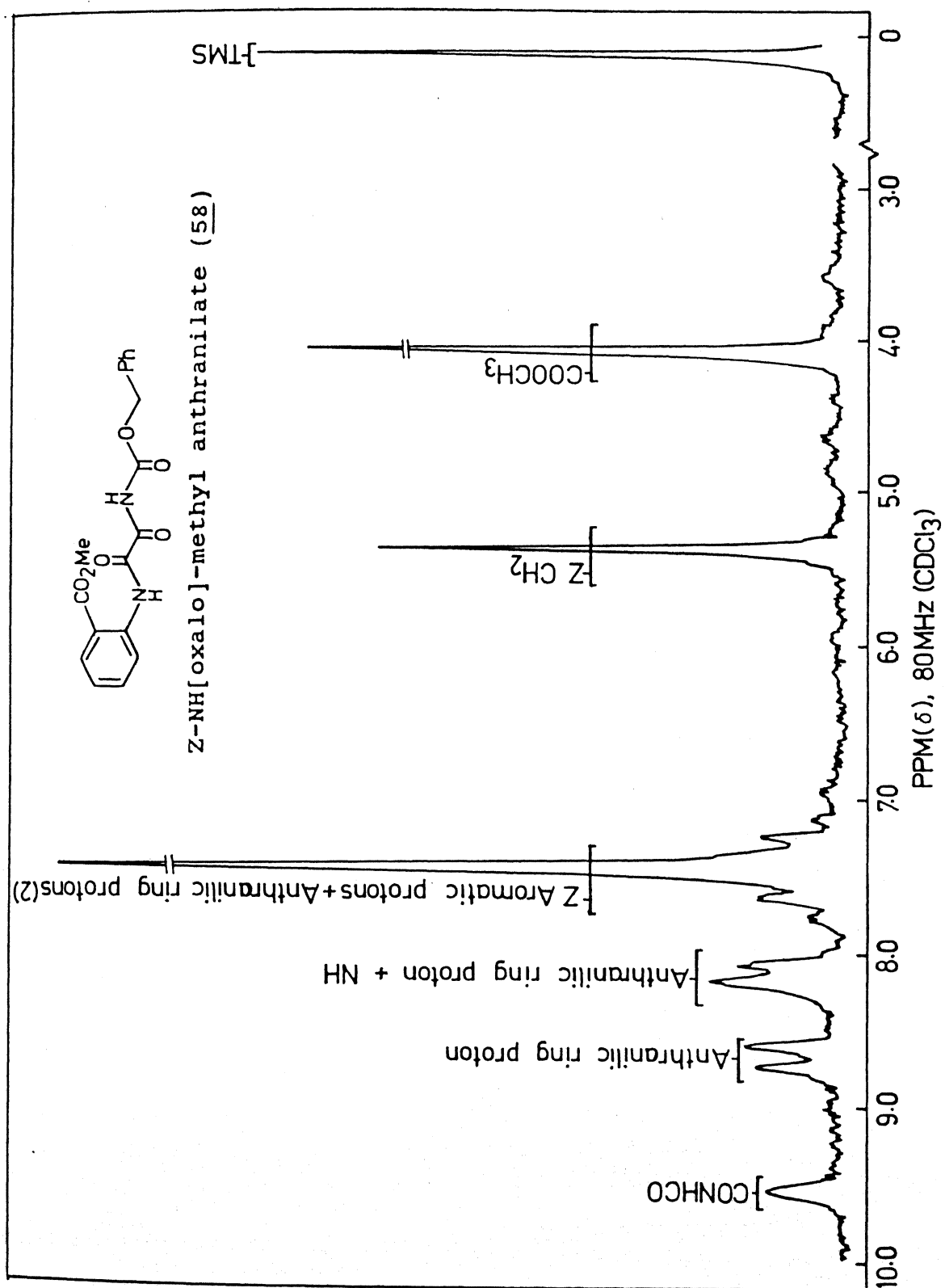


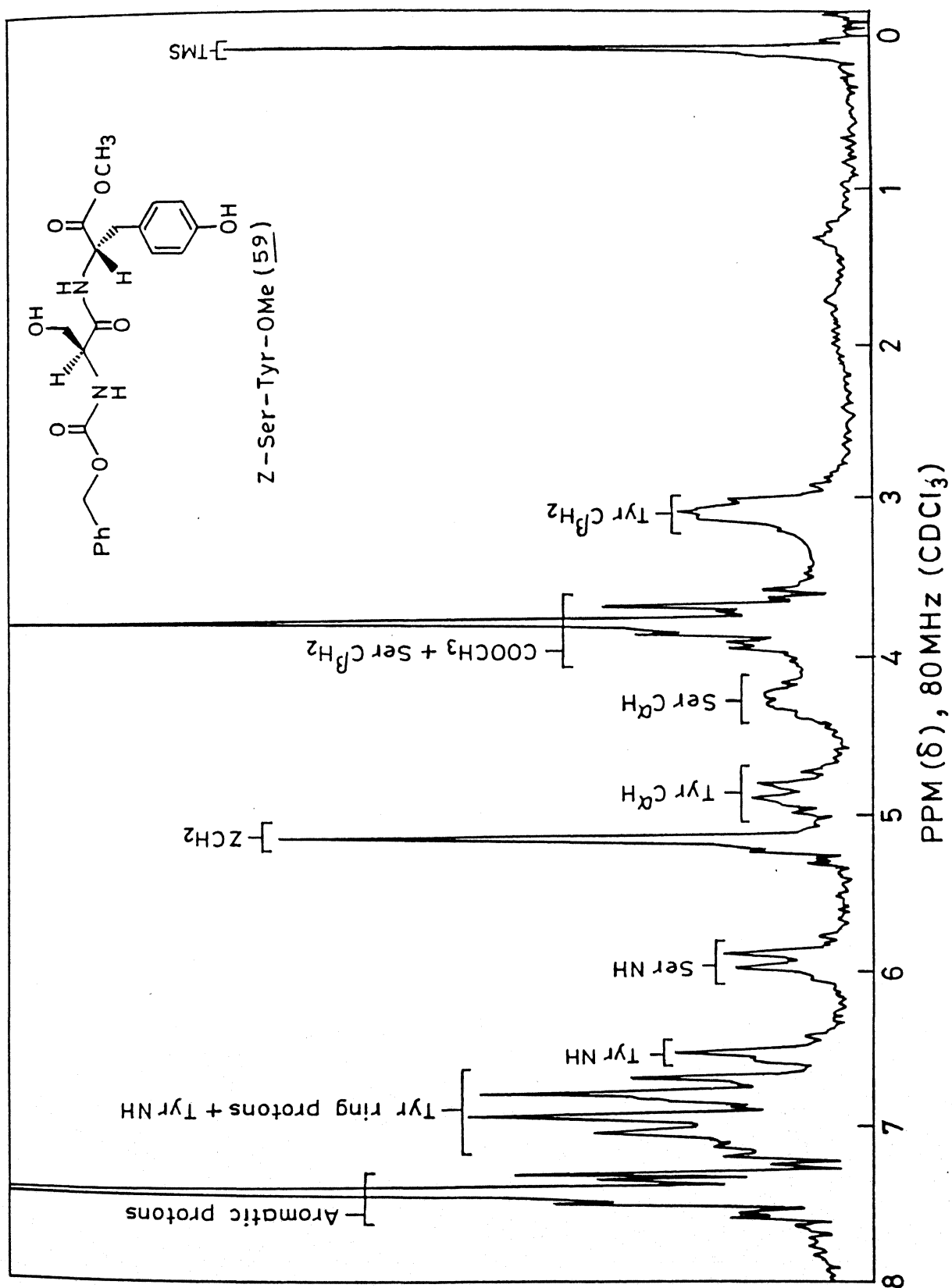


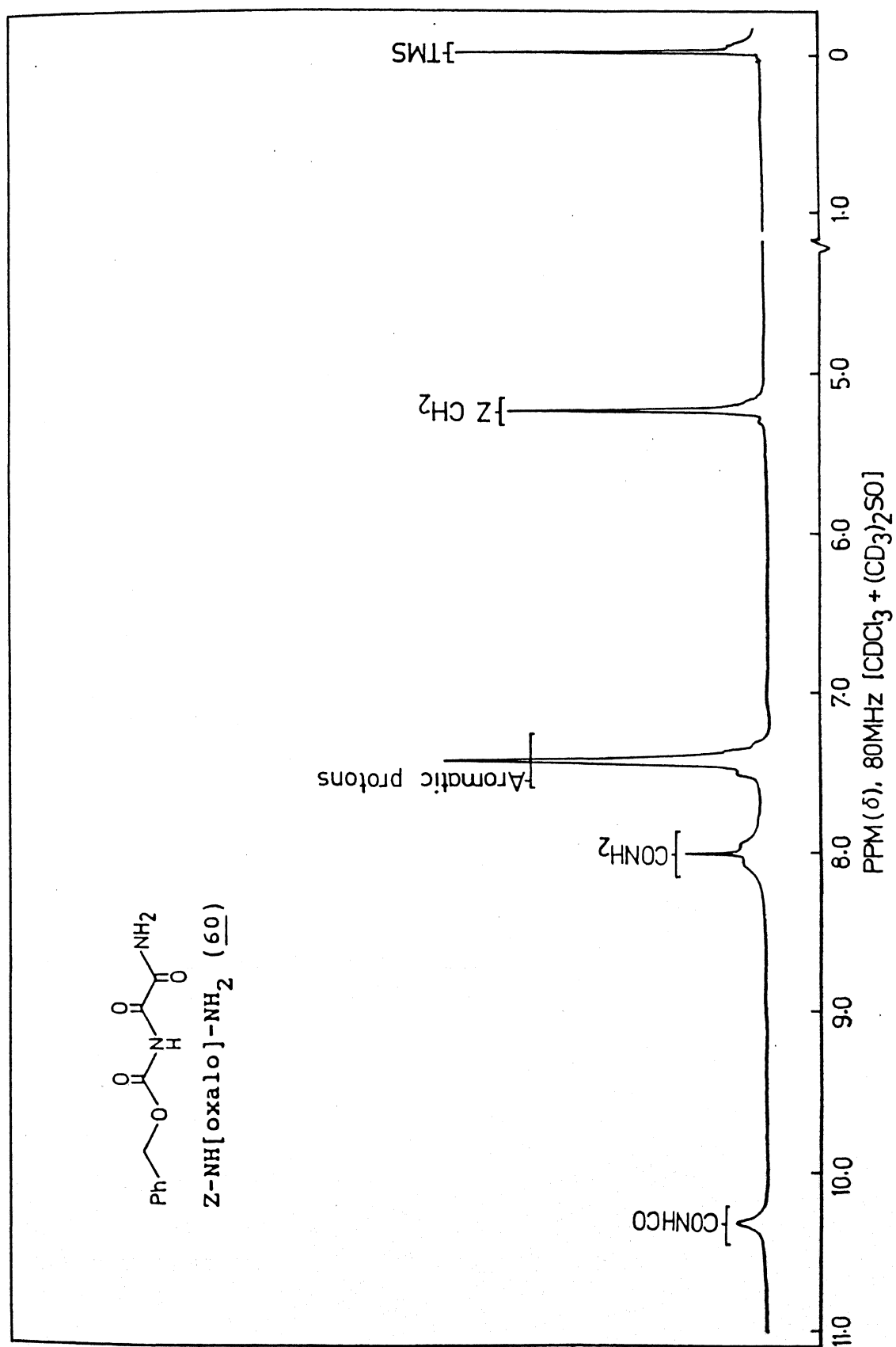


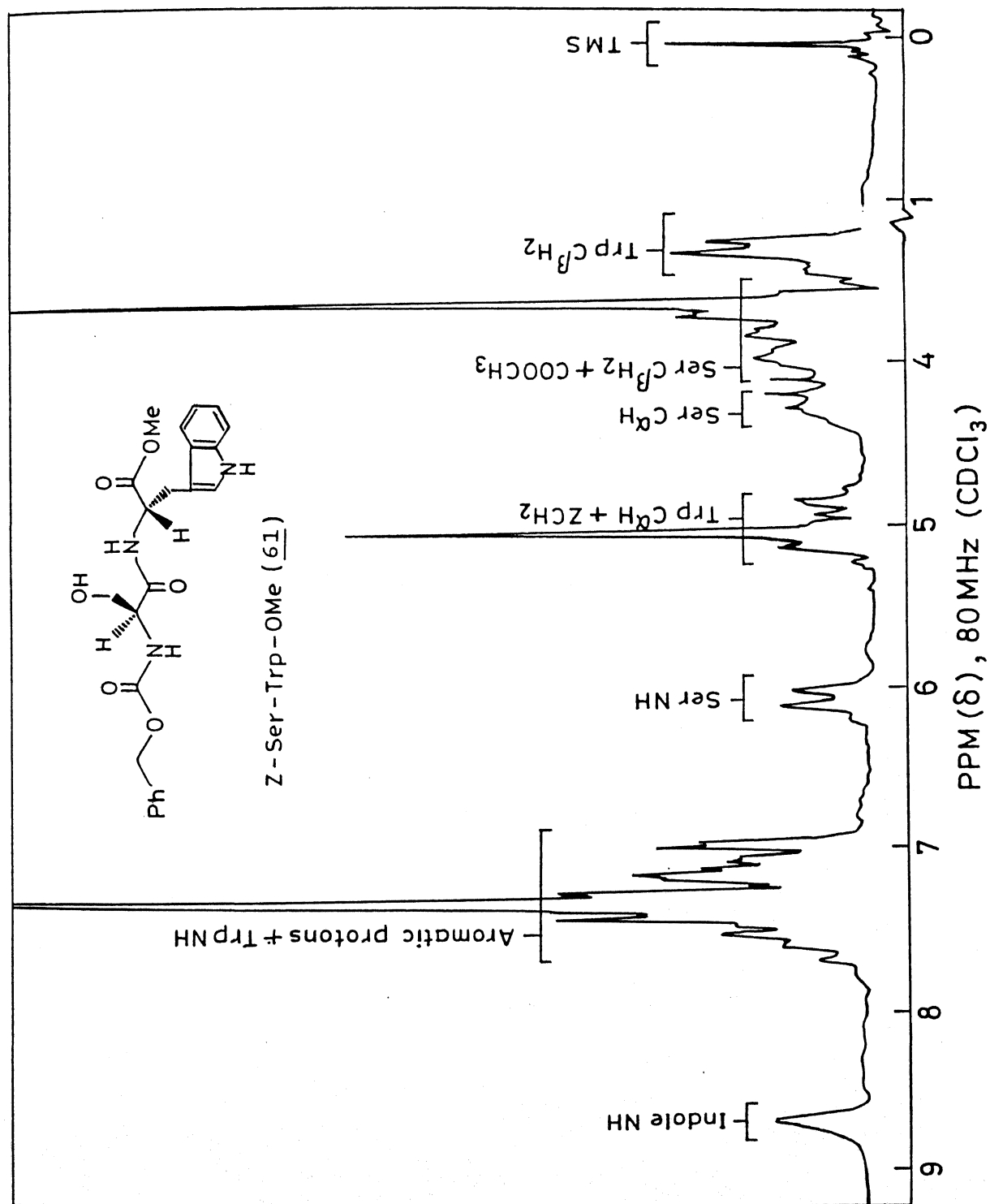


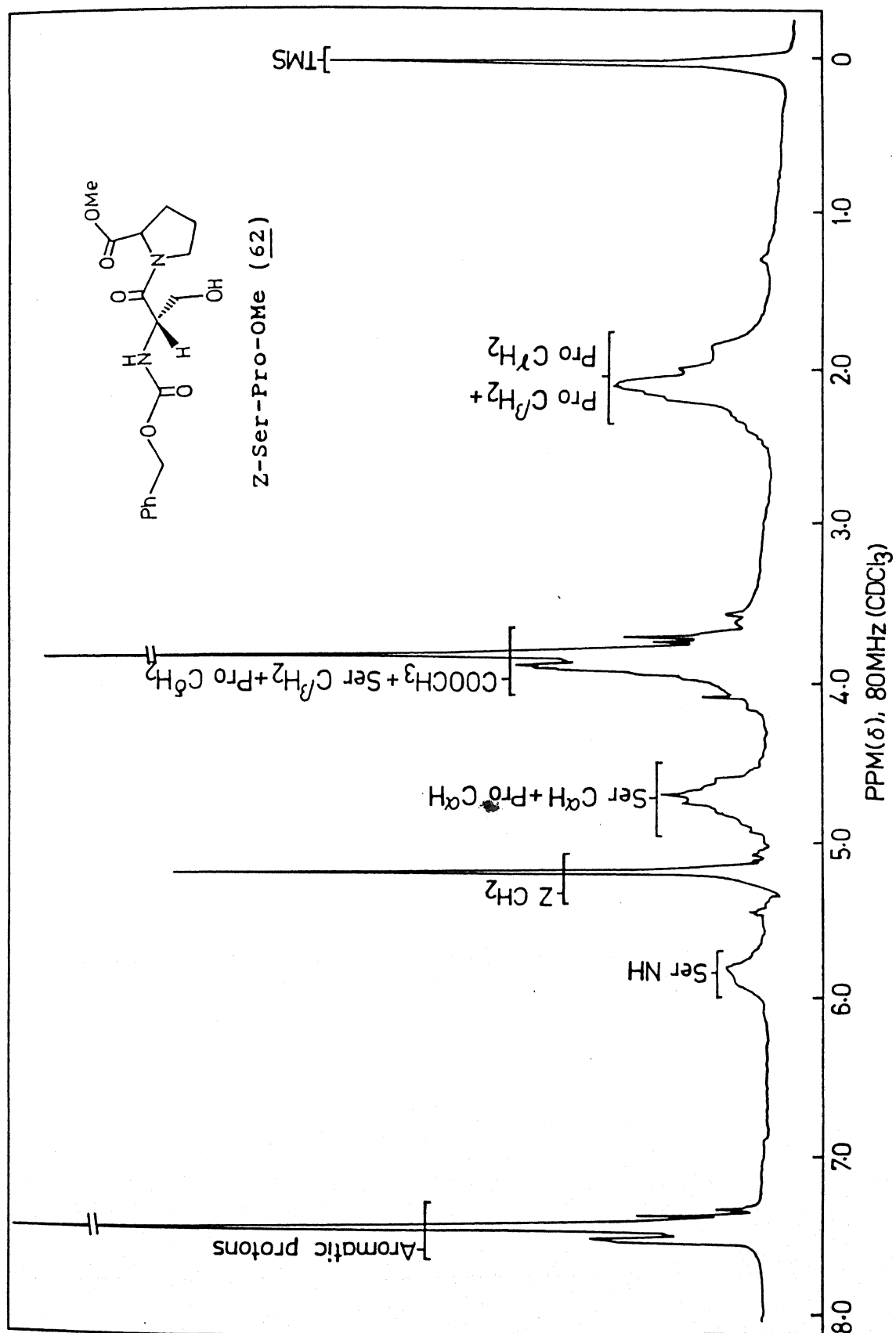
Z-Ser-Methyl anthranilate (57)

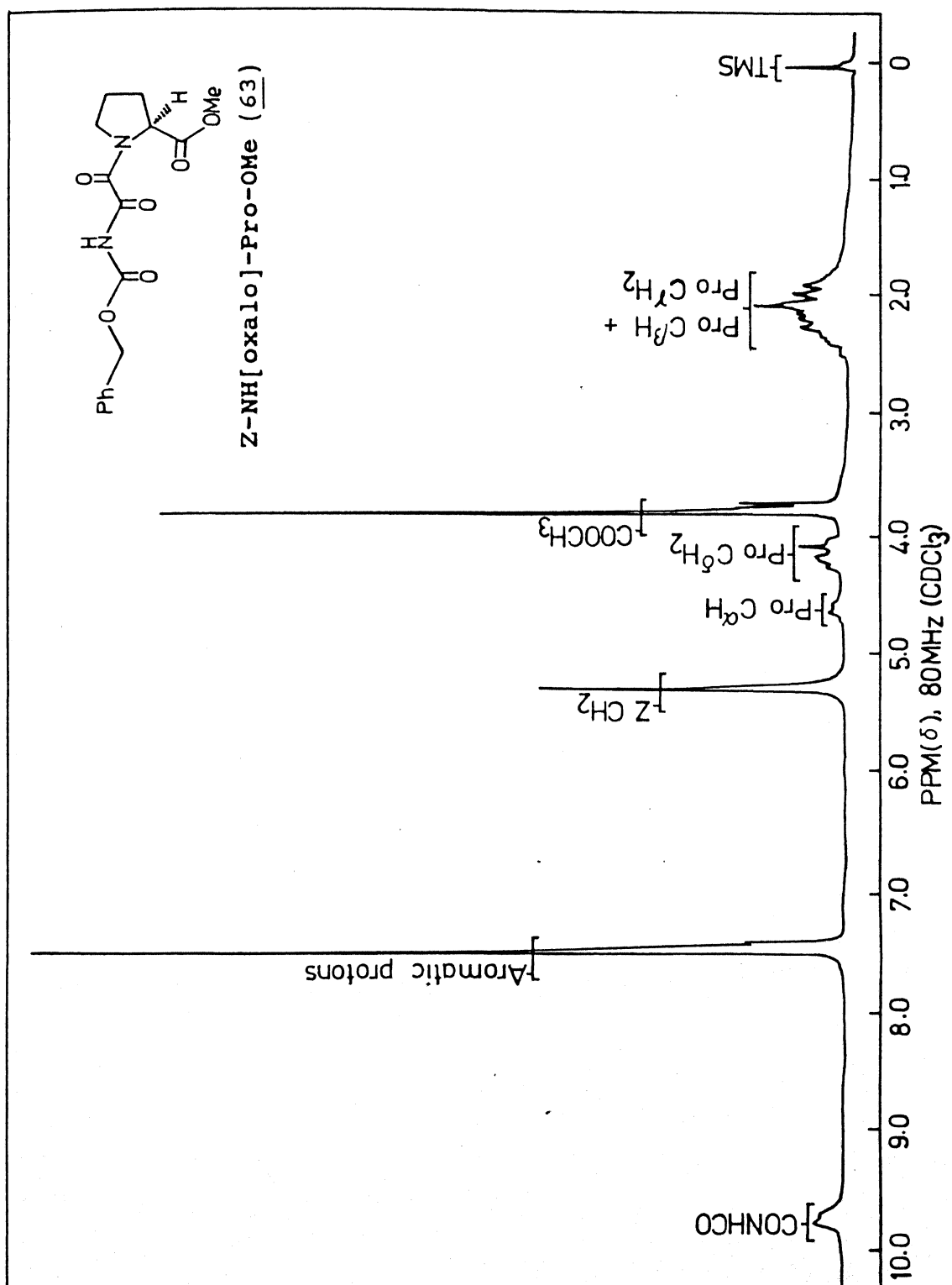


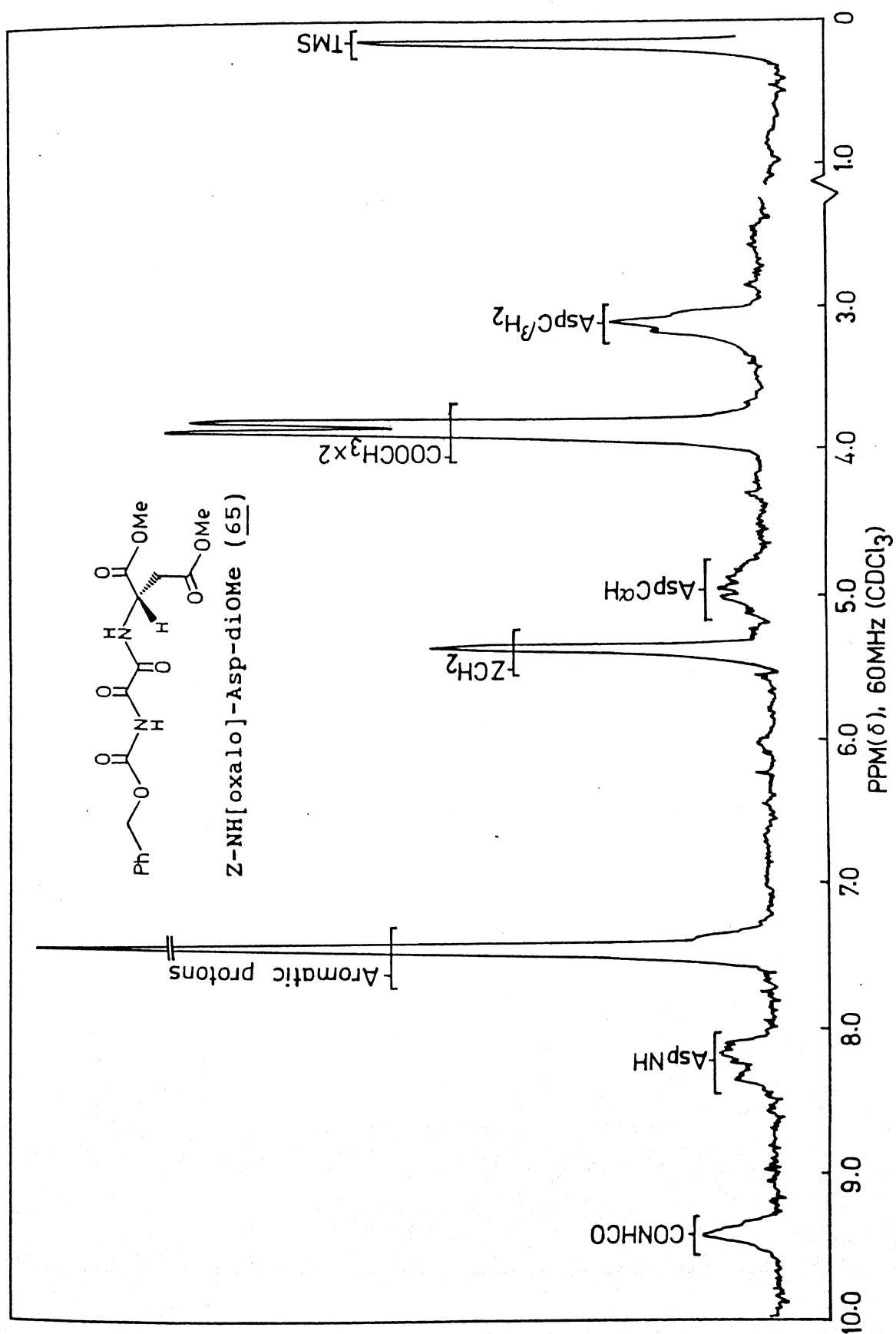


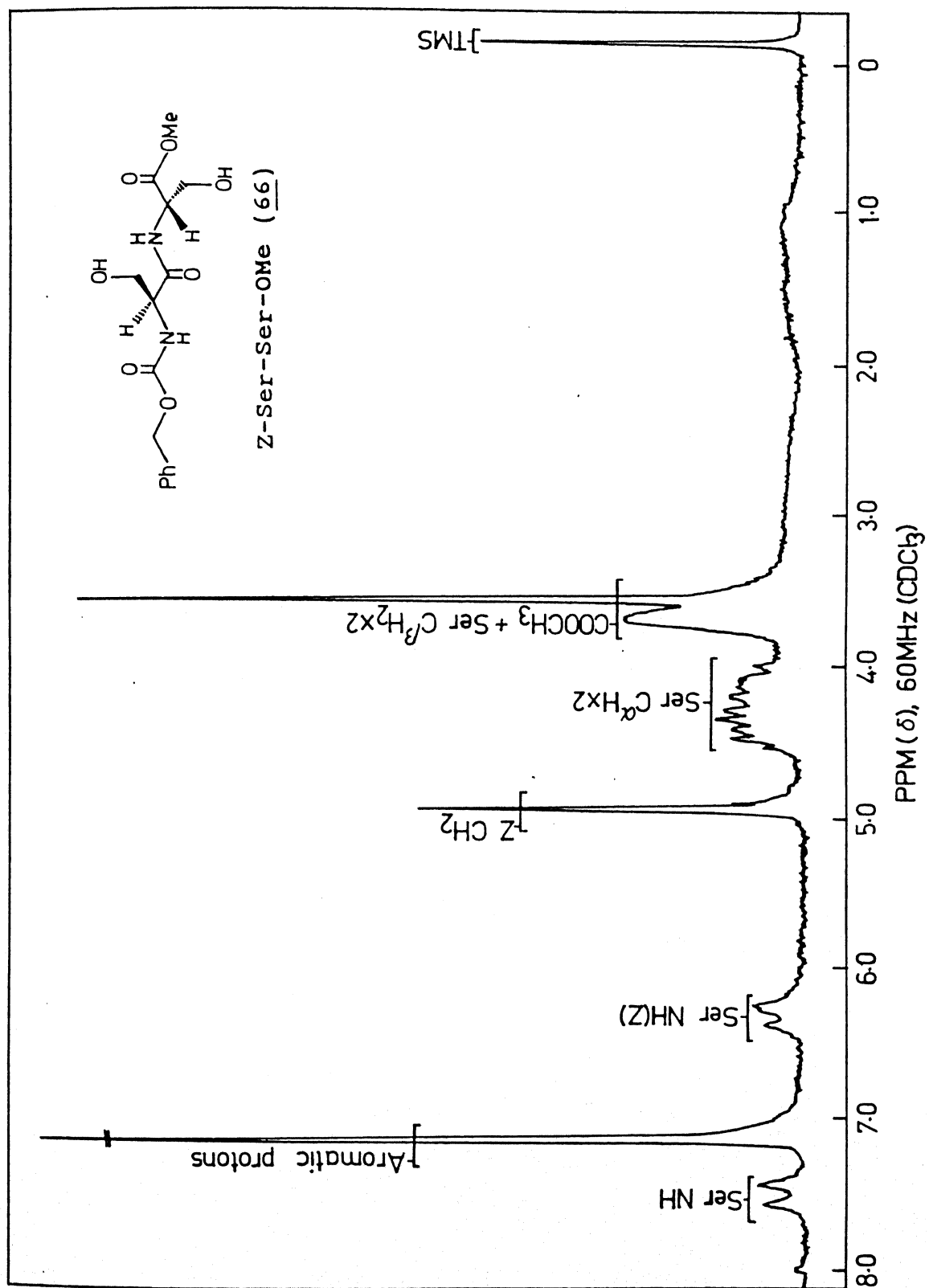


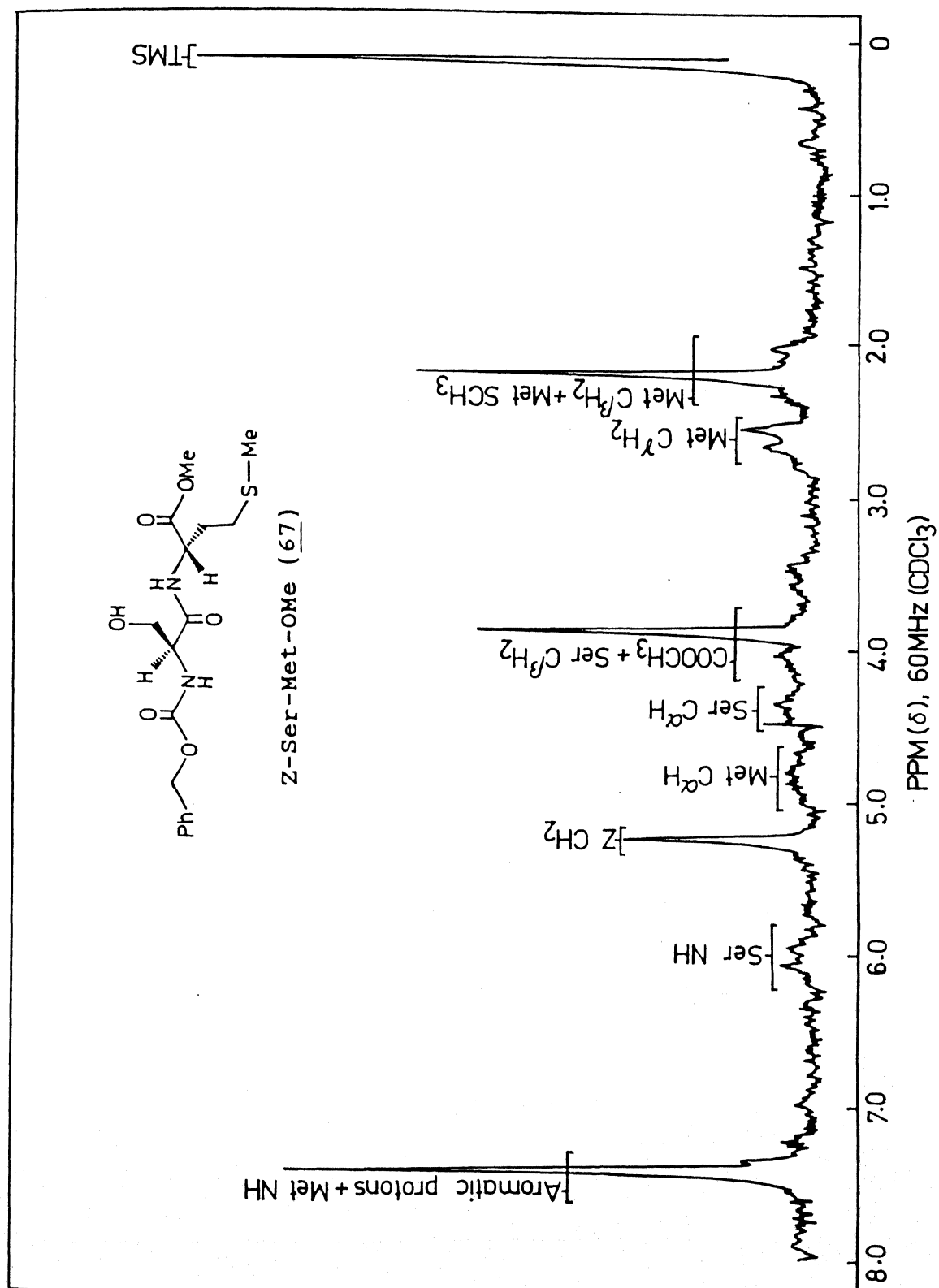


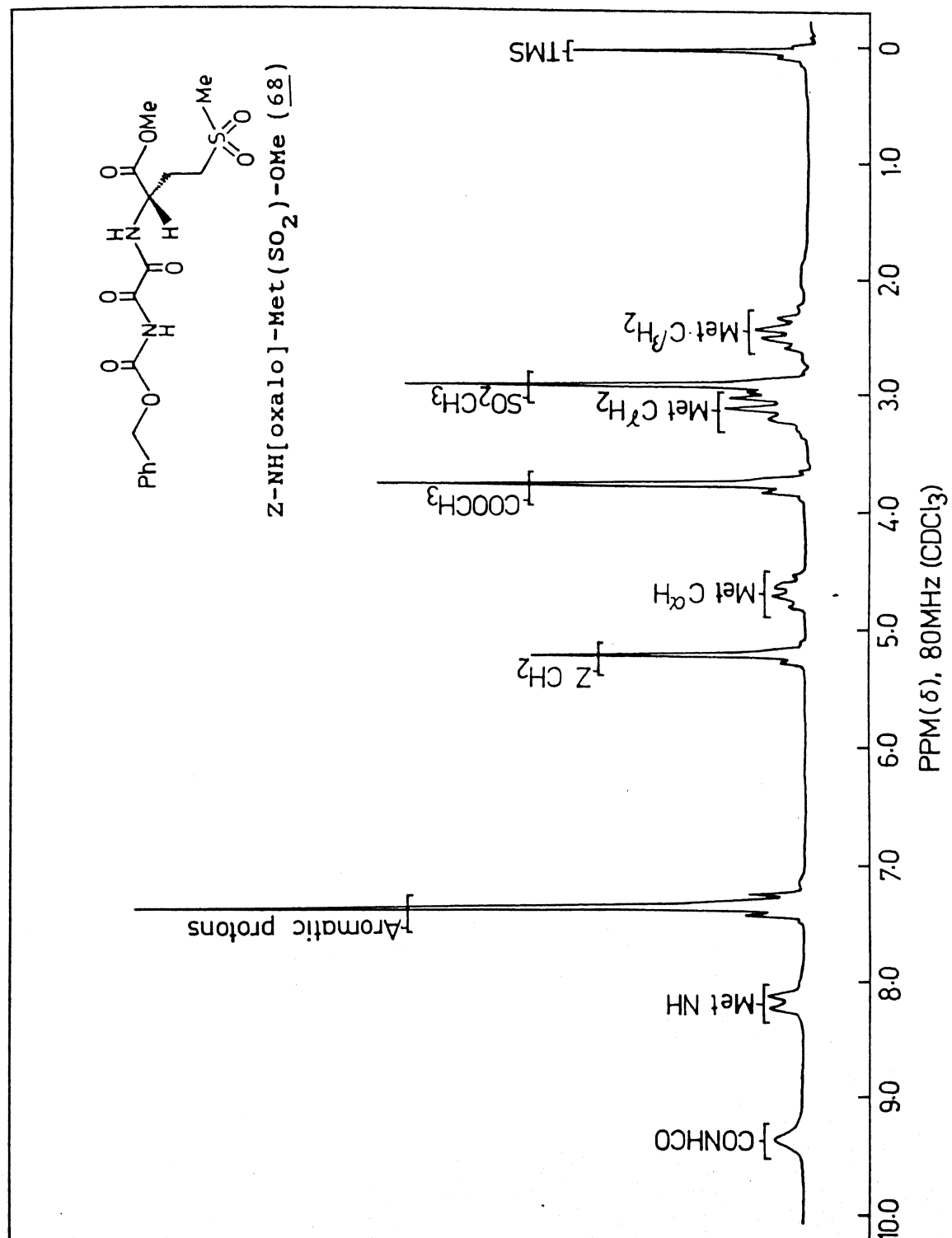


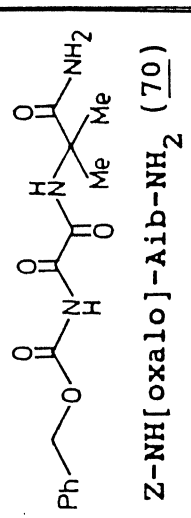


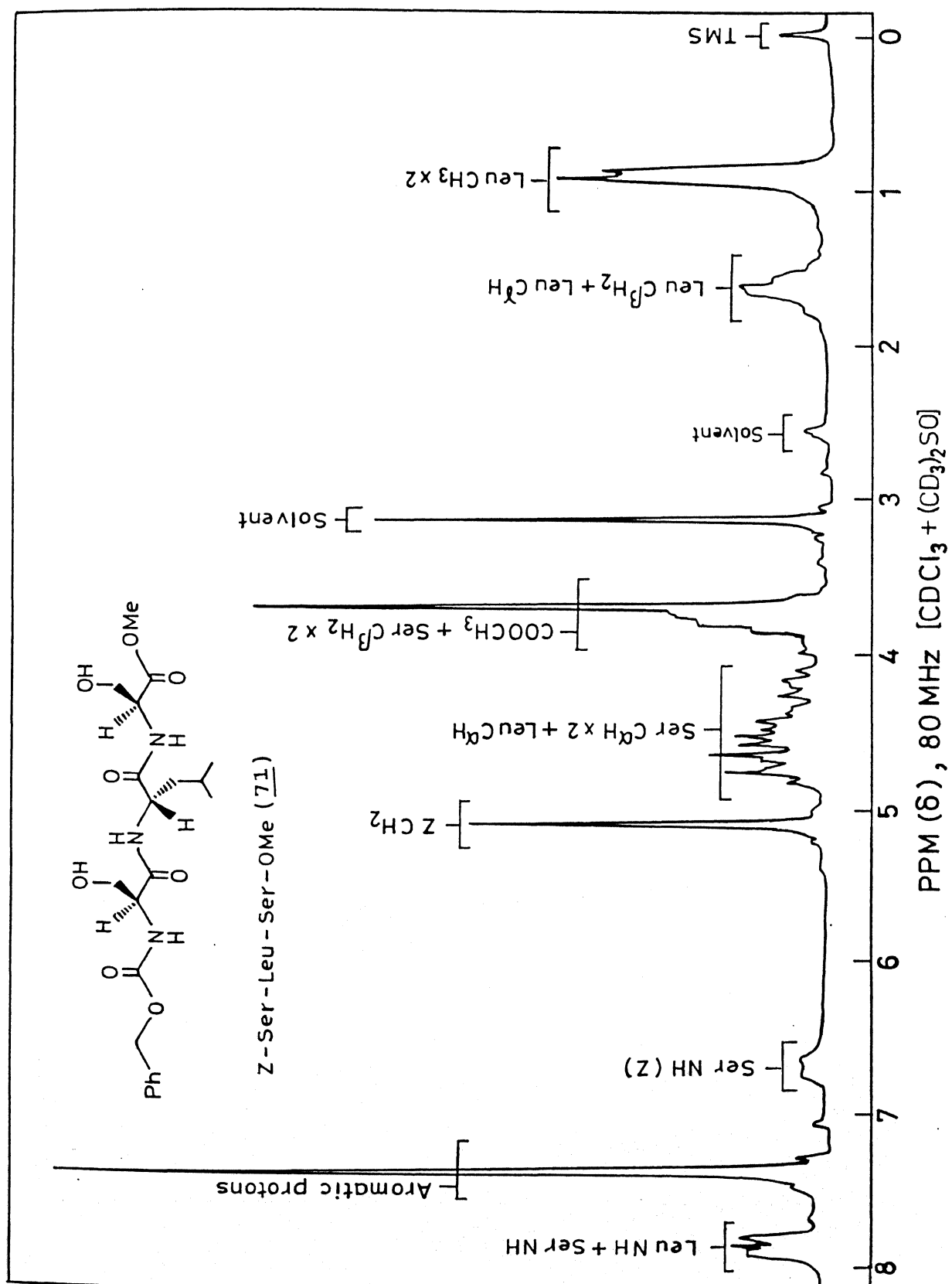


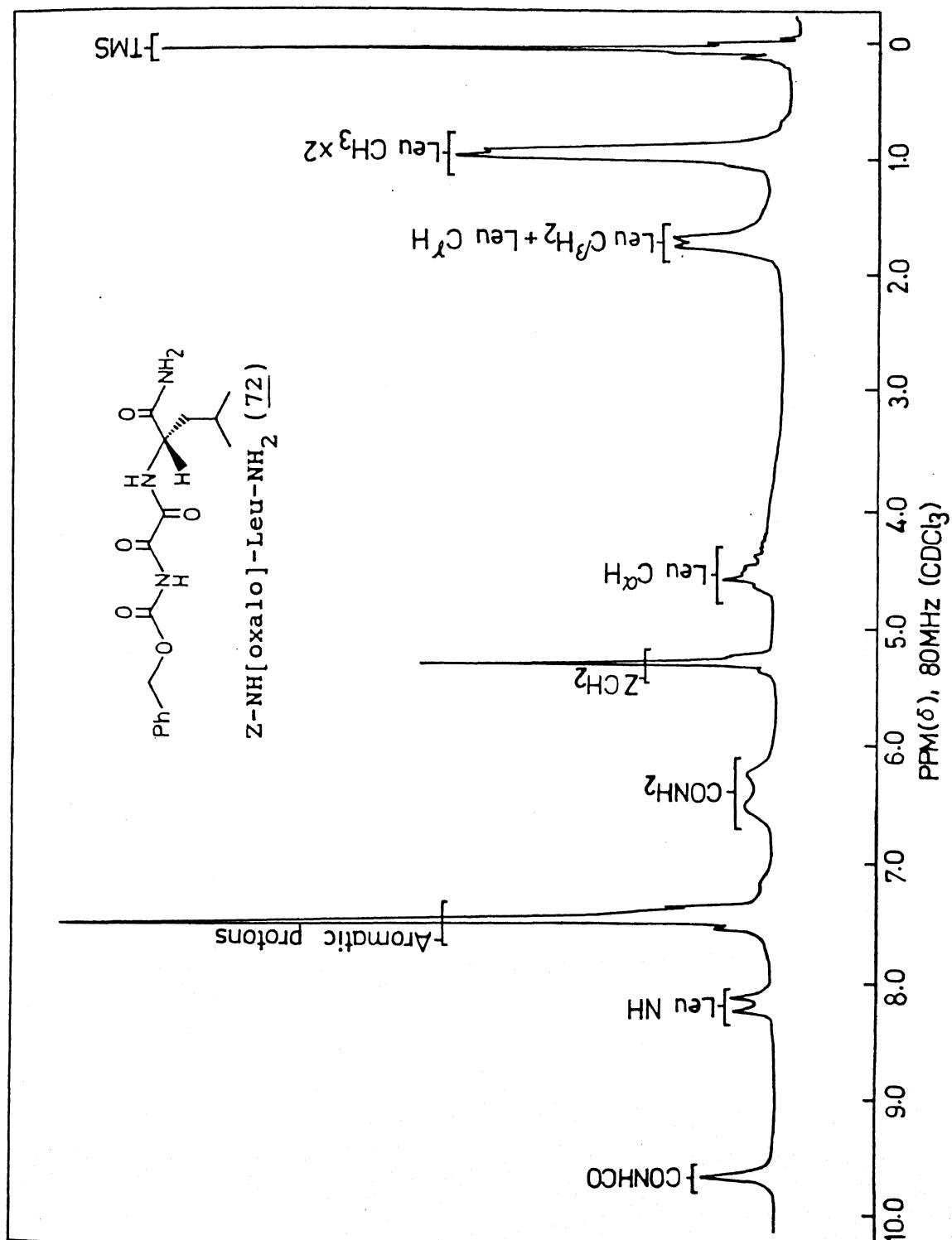


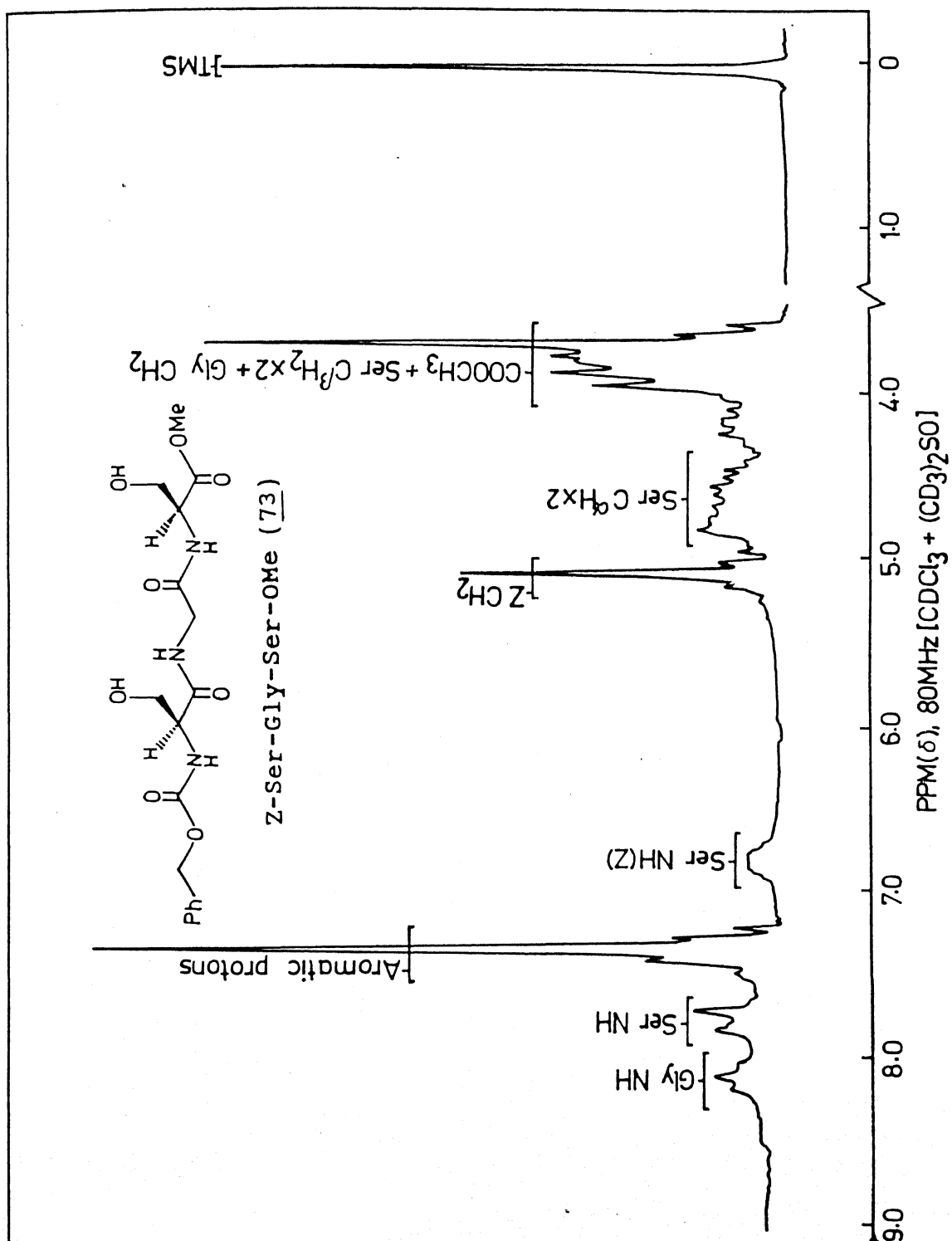


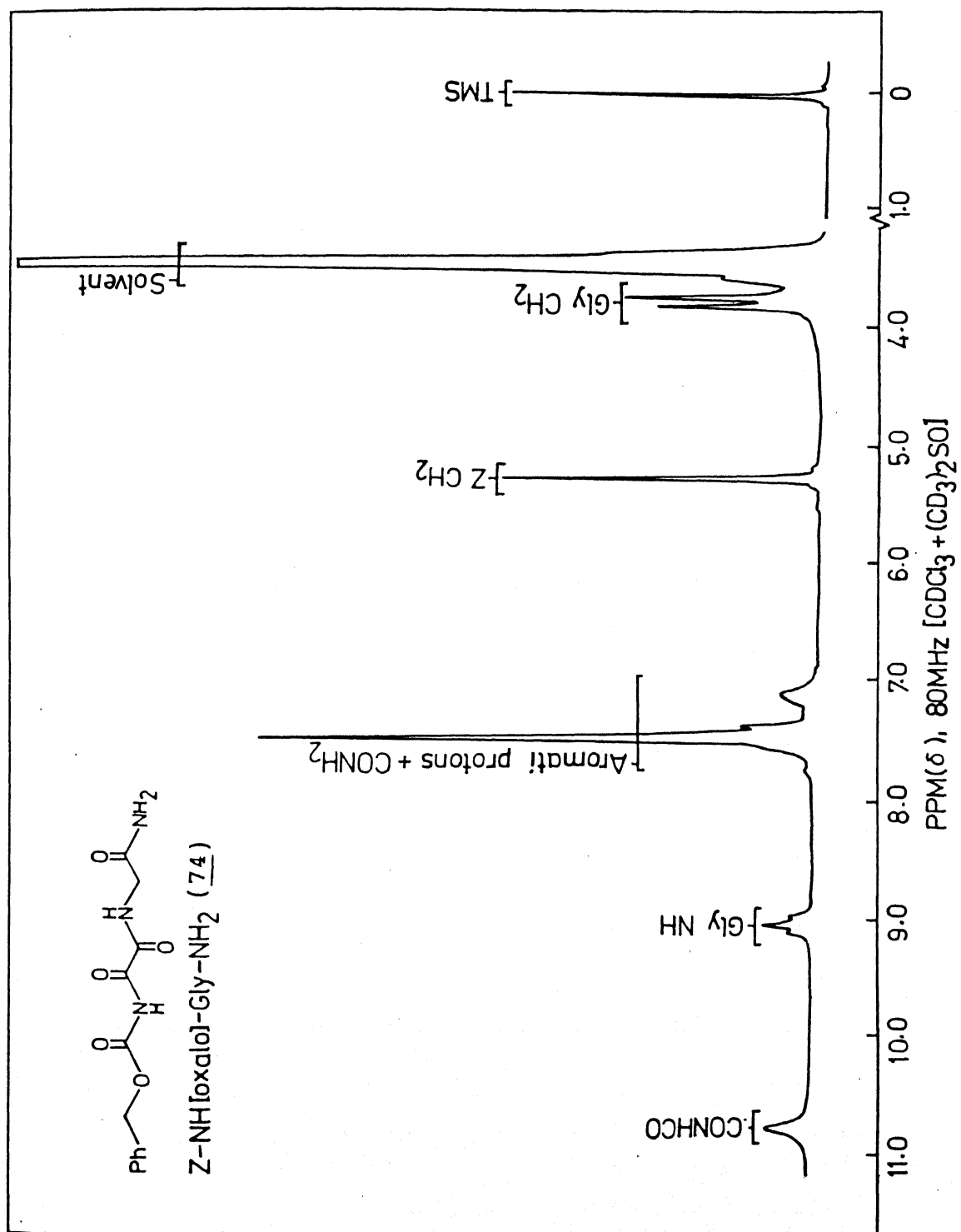


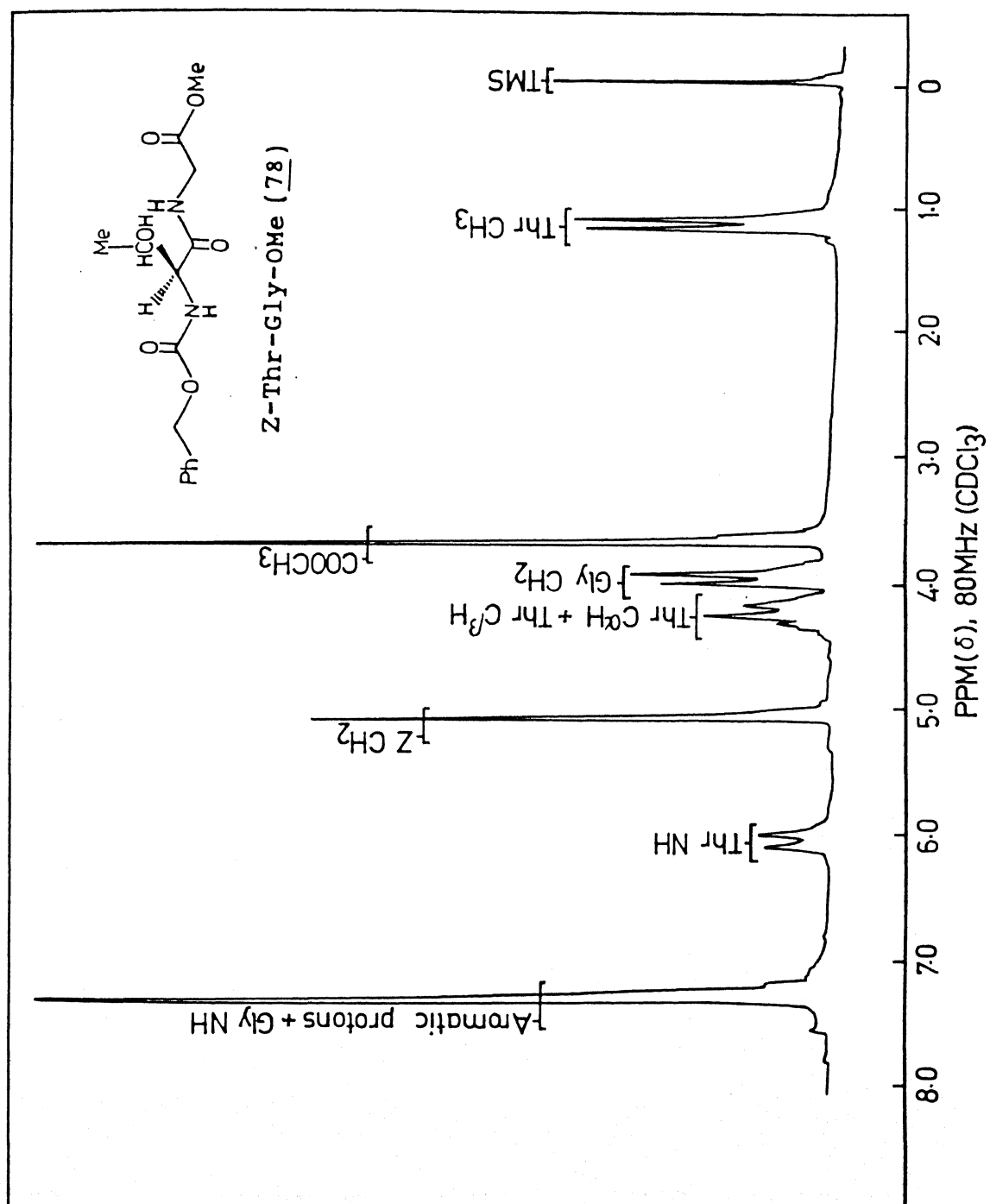


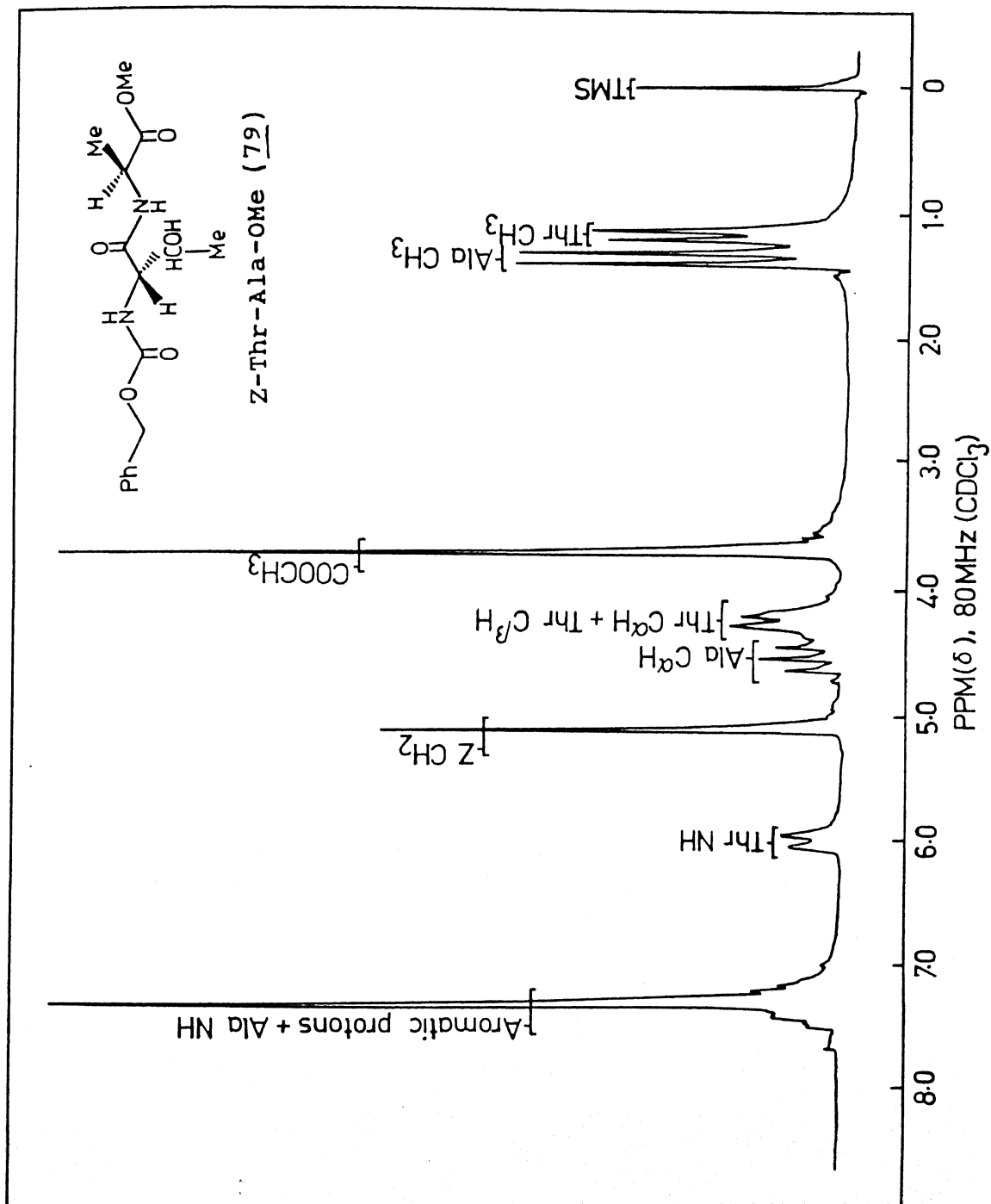


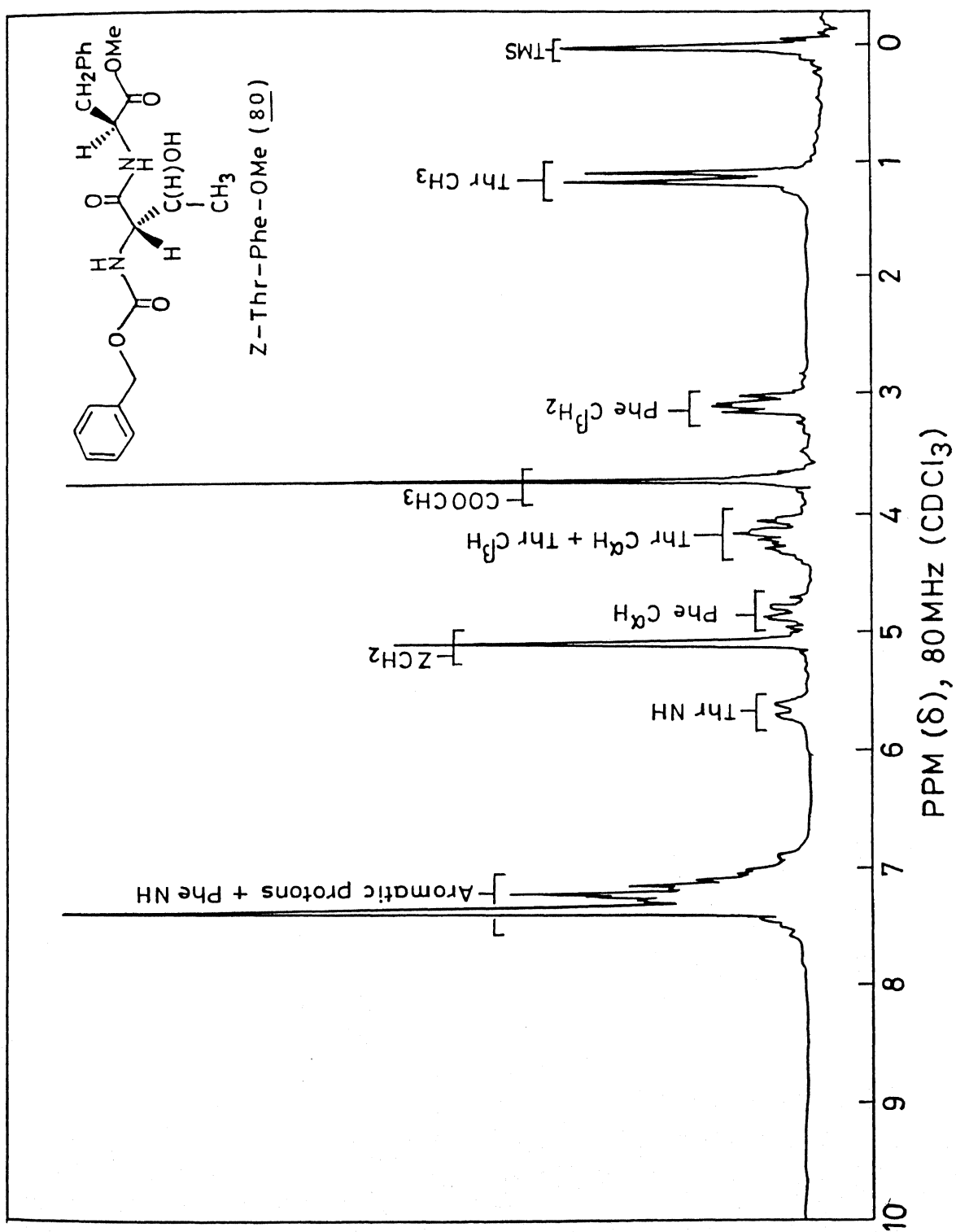


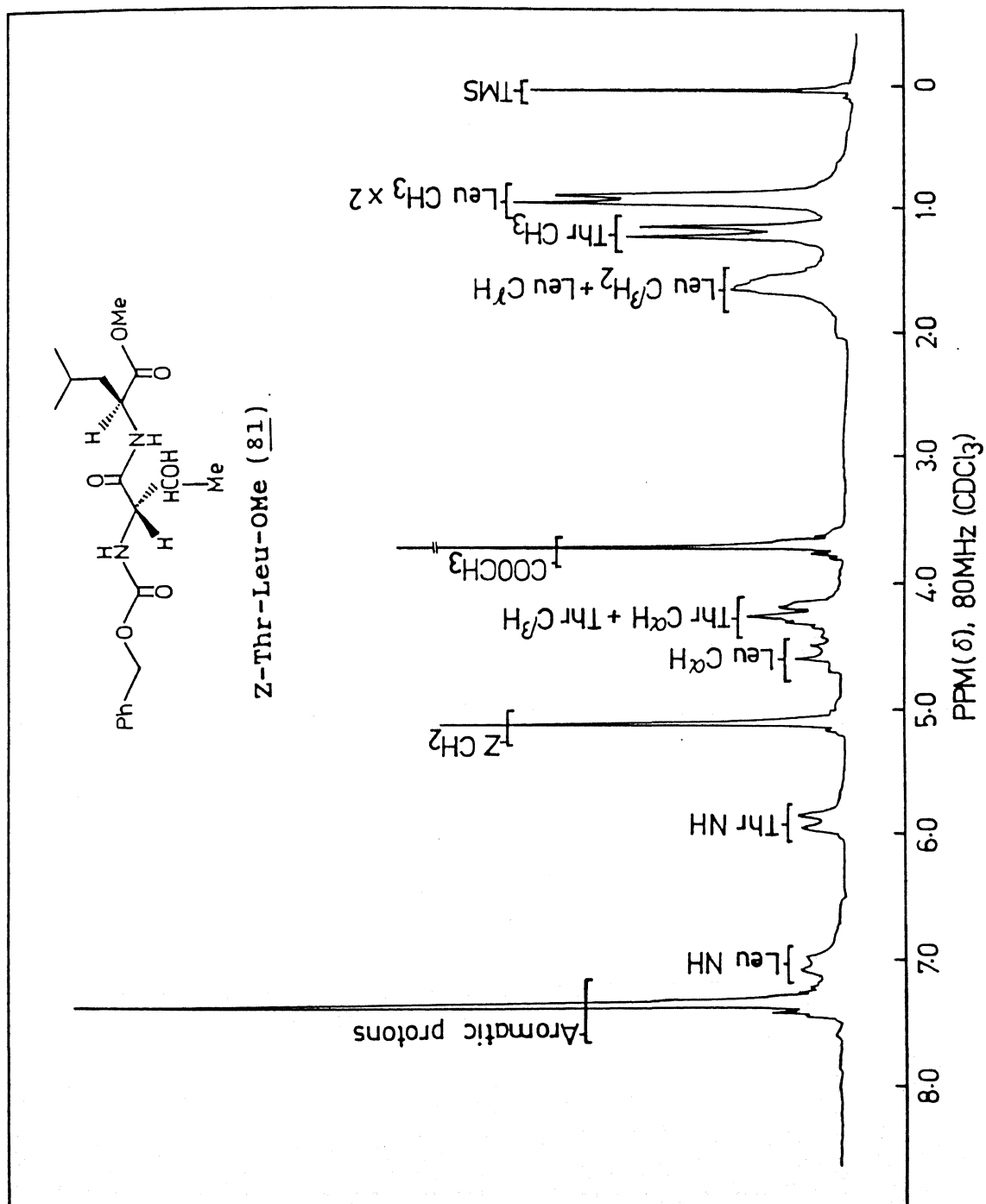


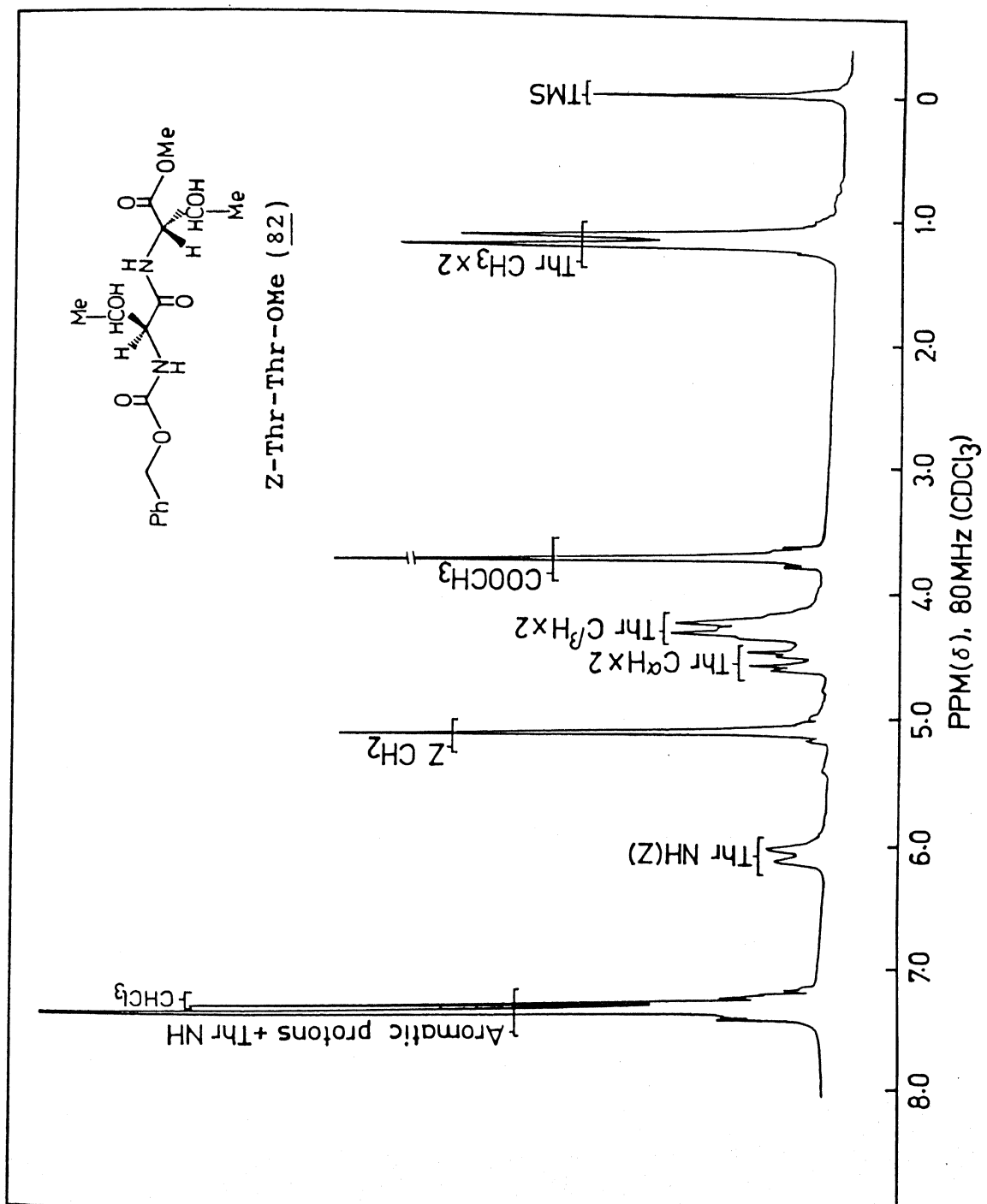


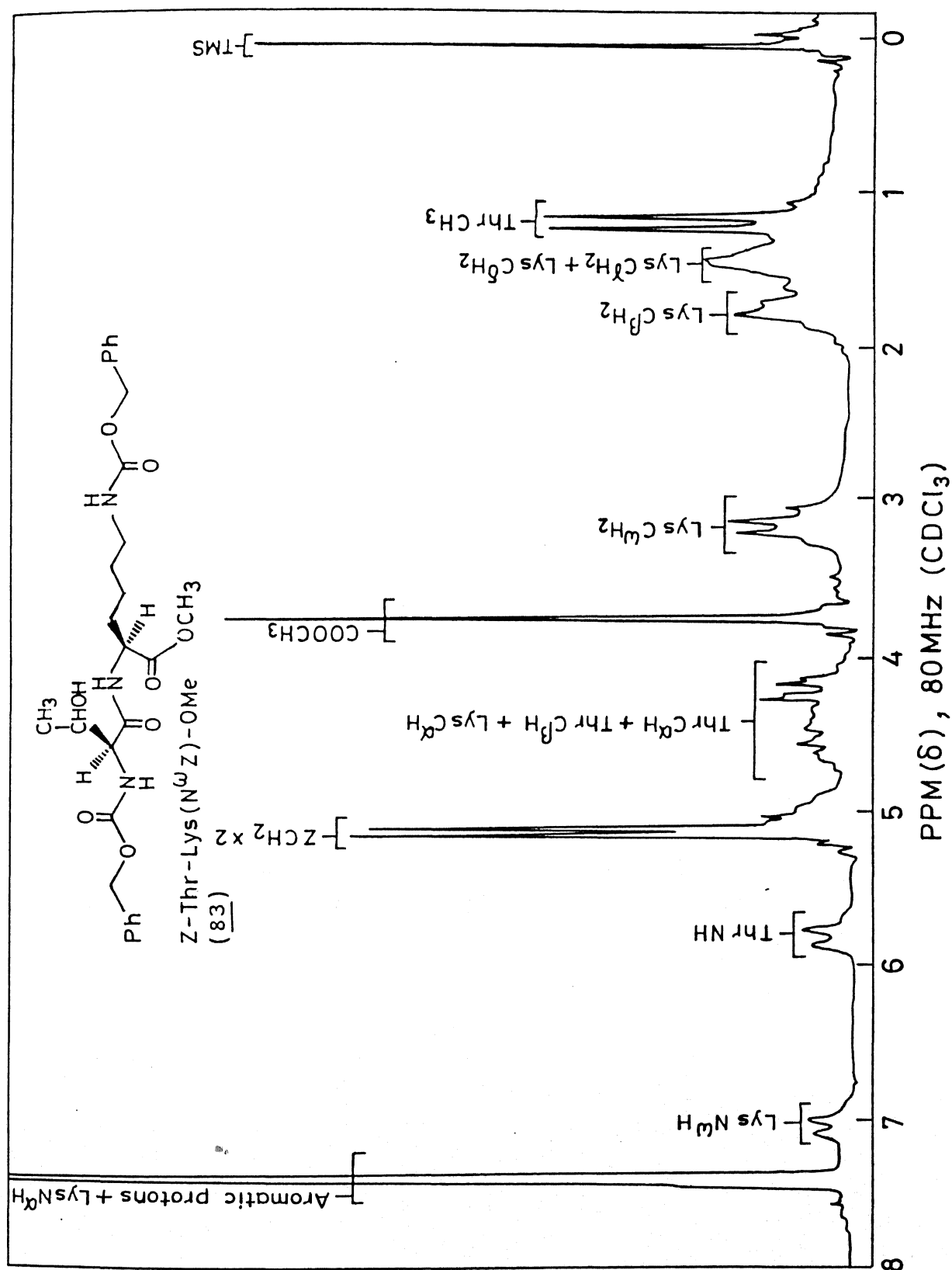


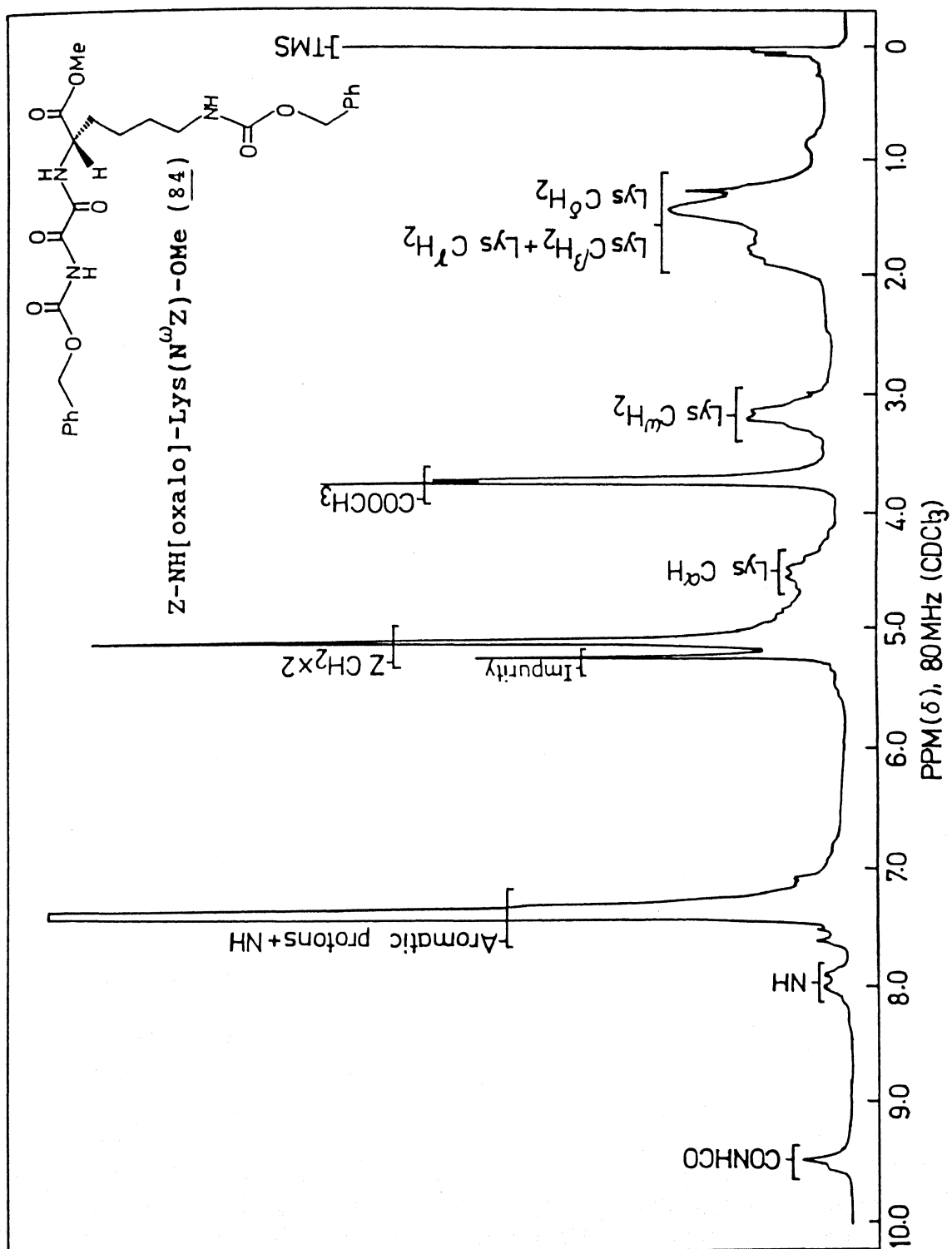


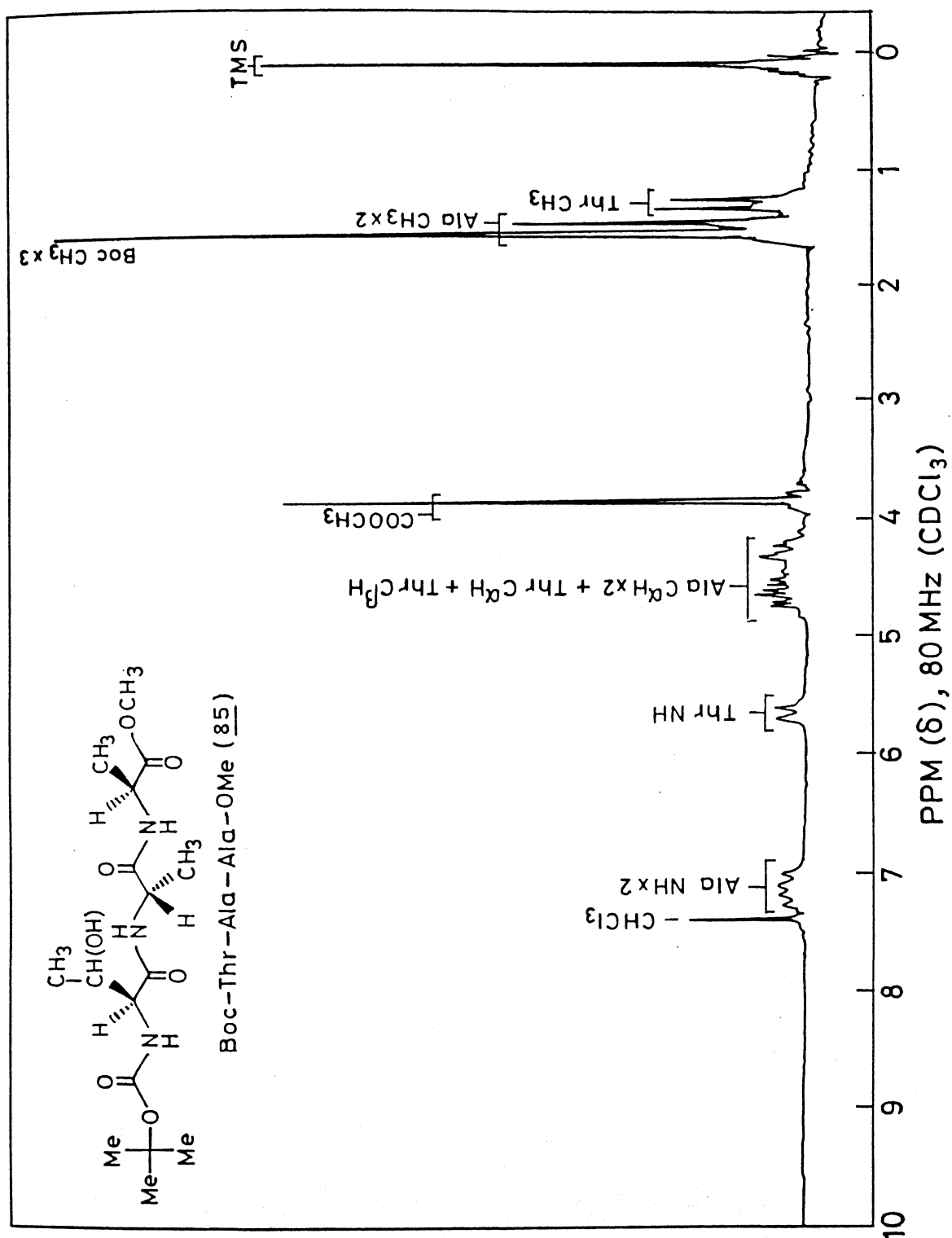


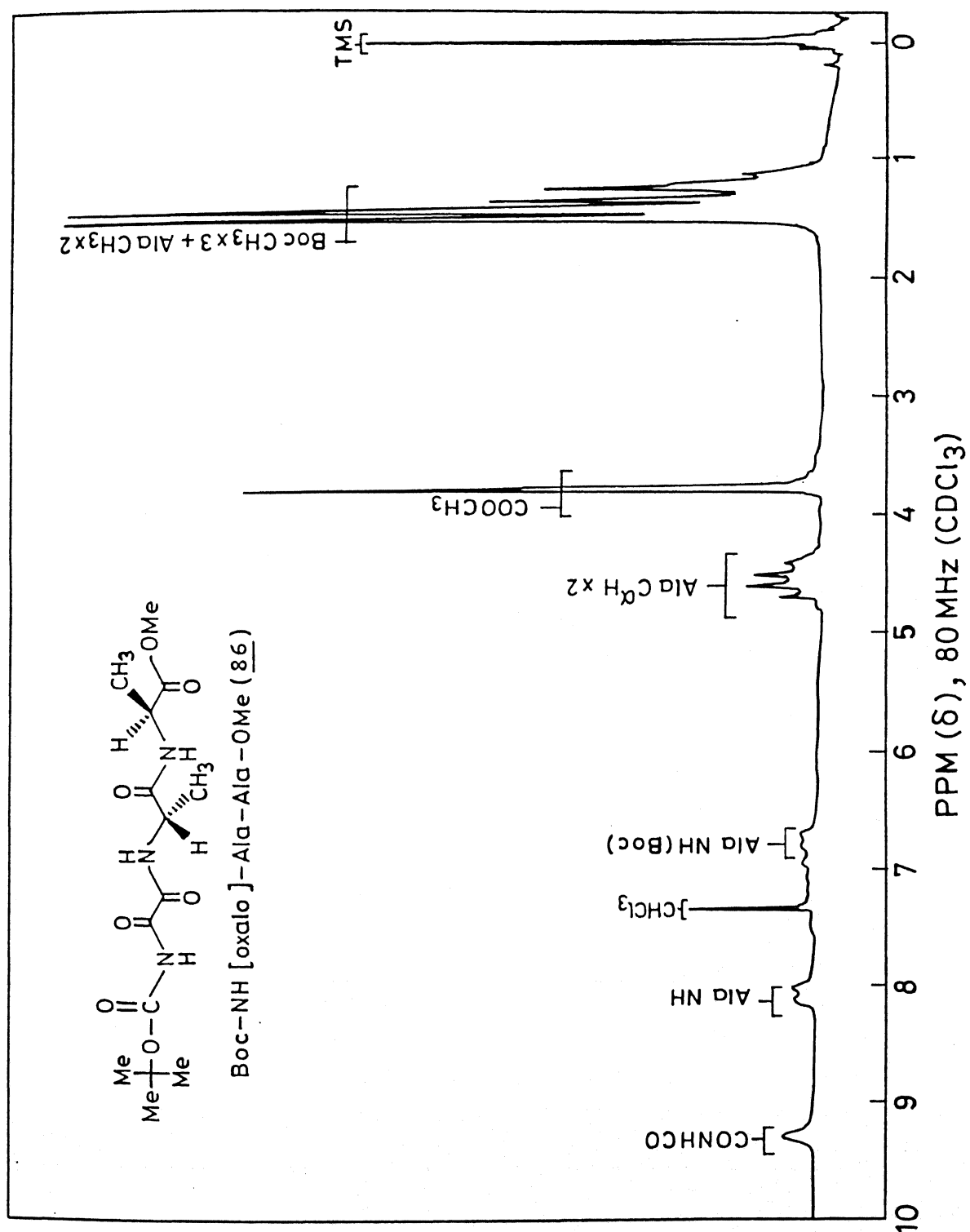


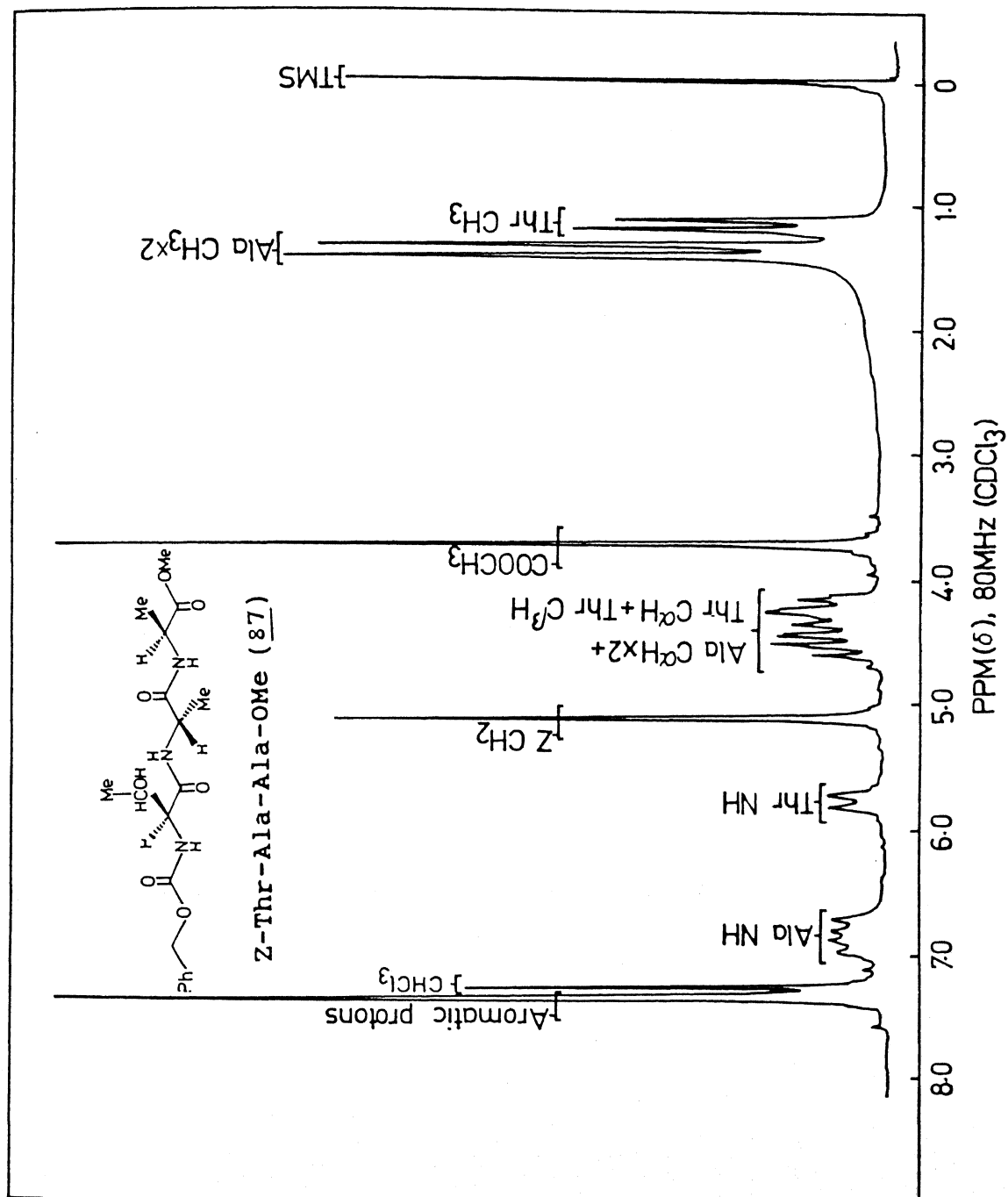


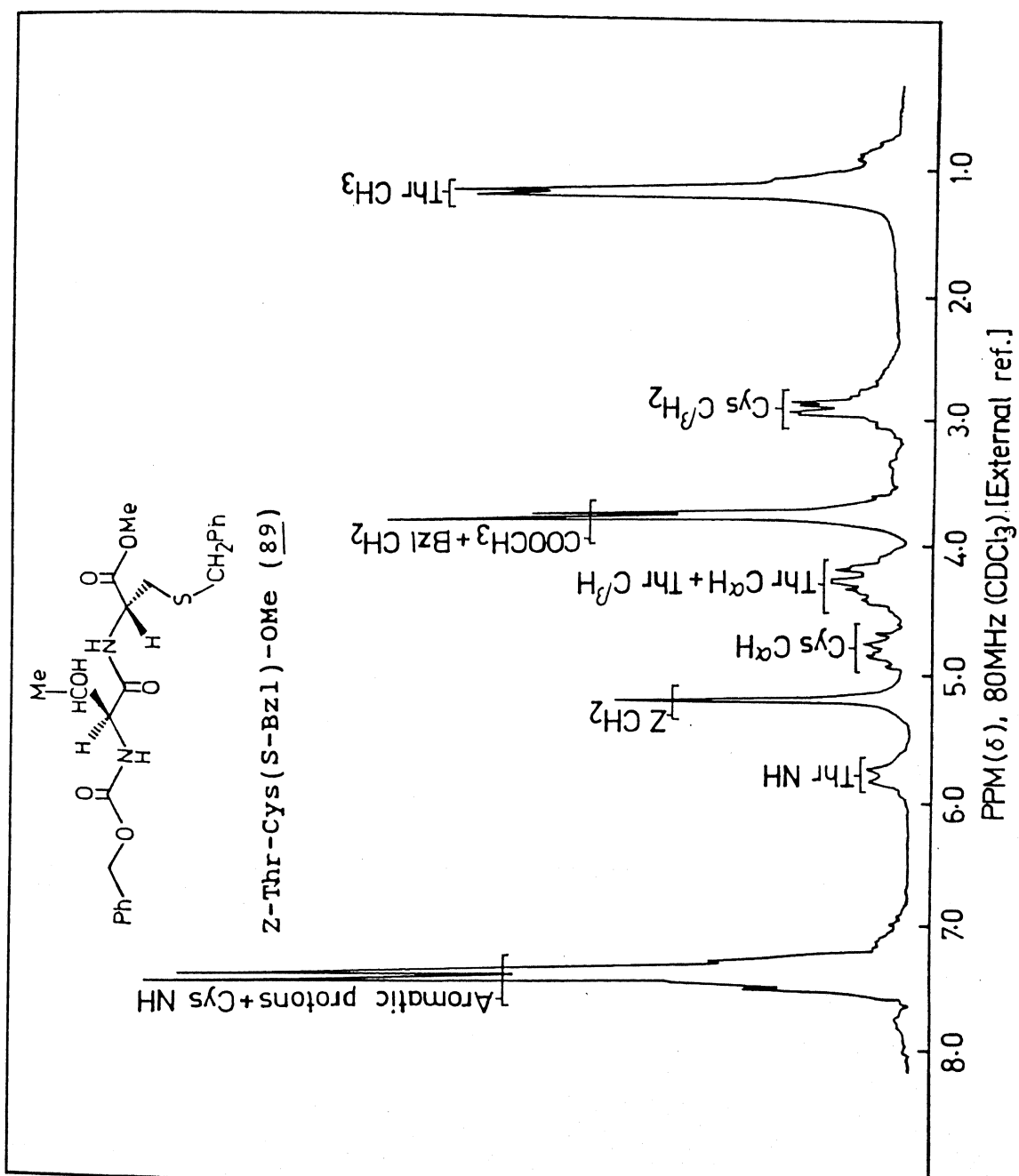


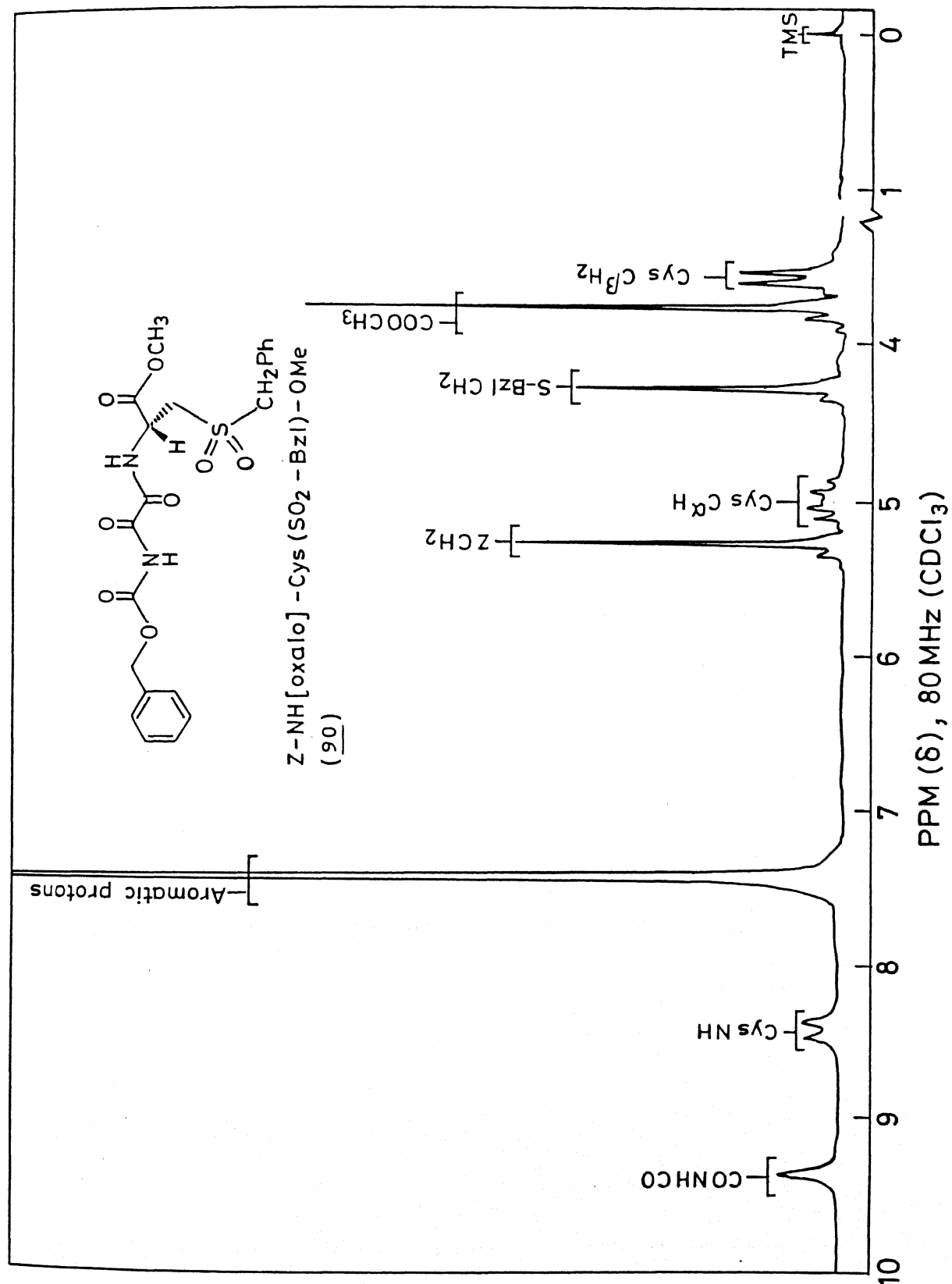


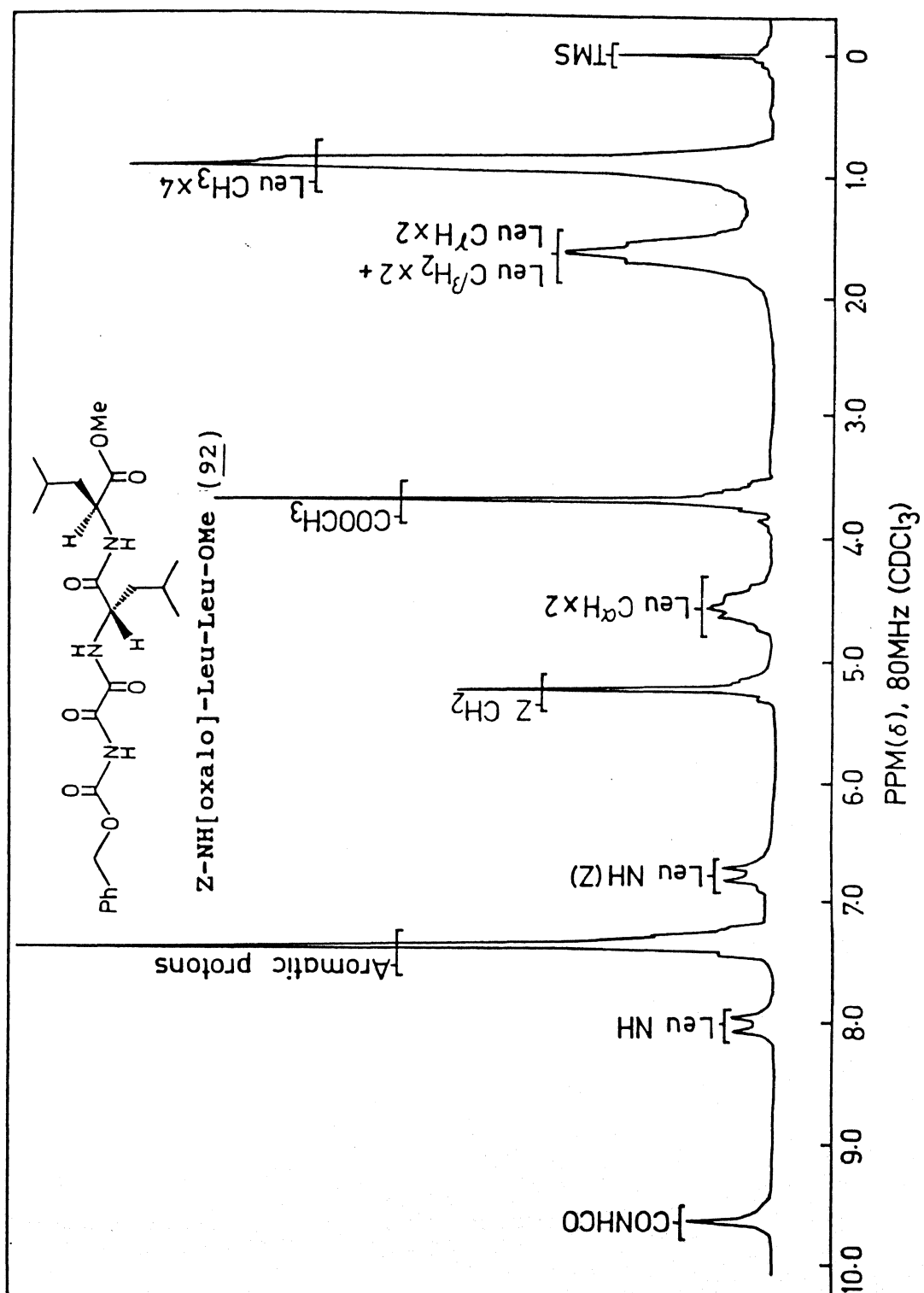


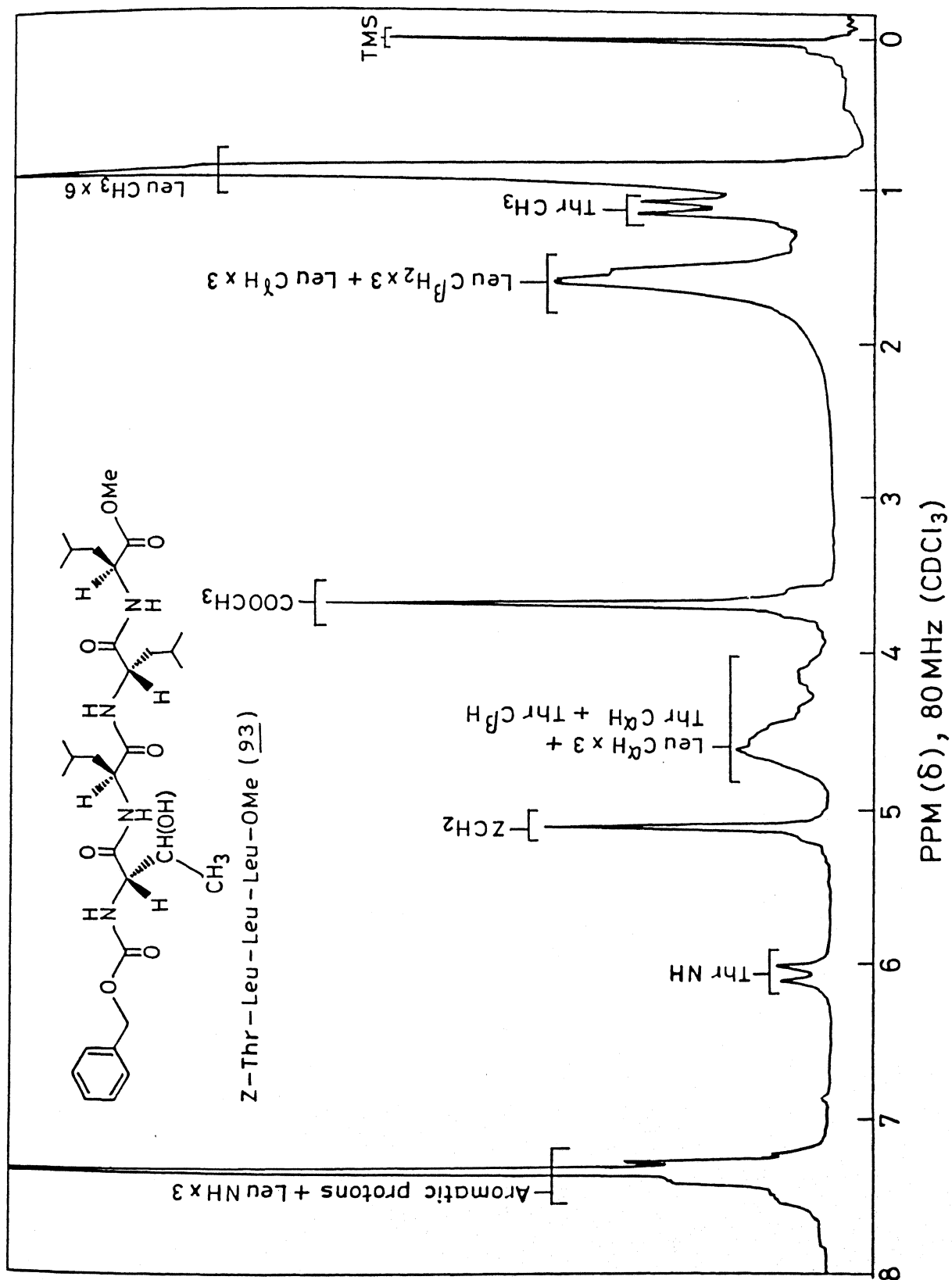


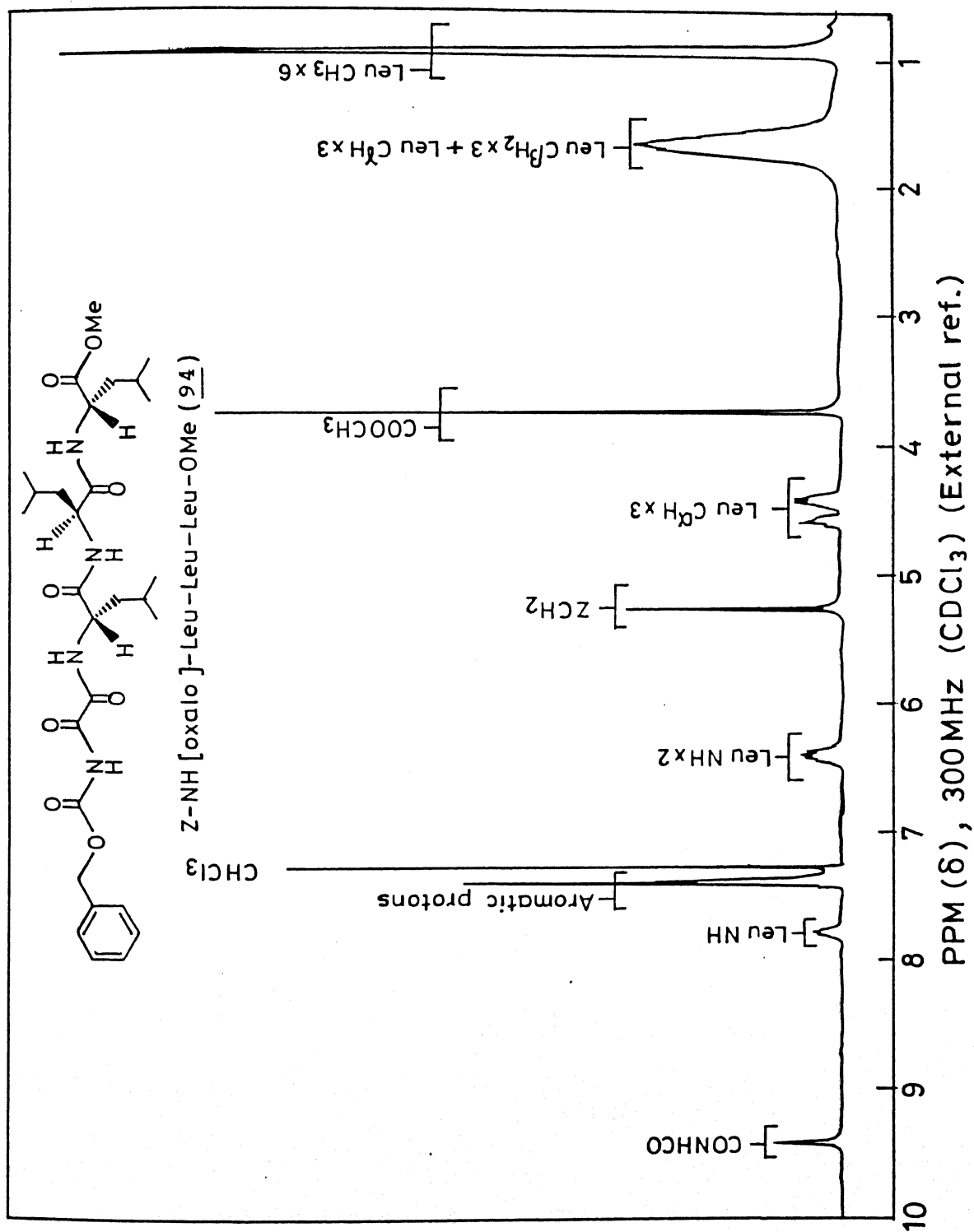


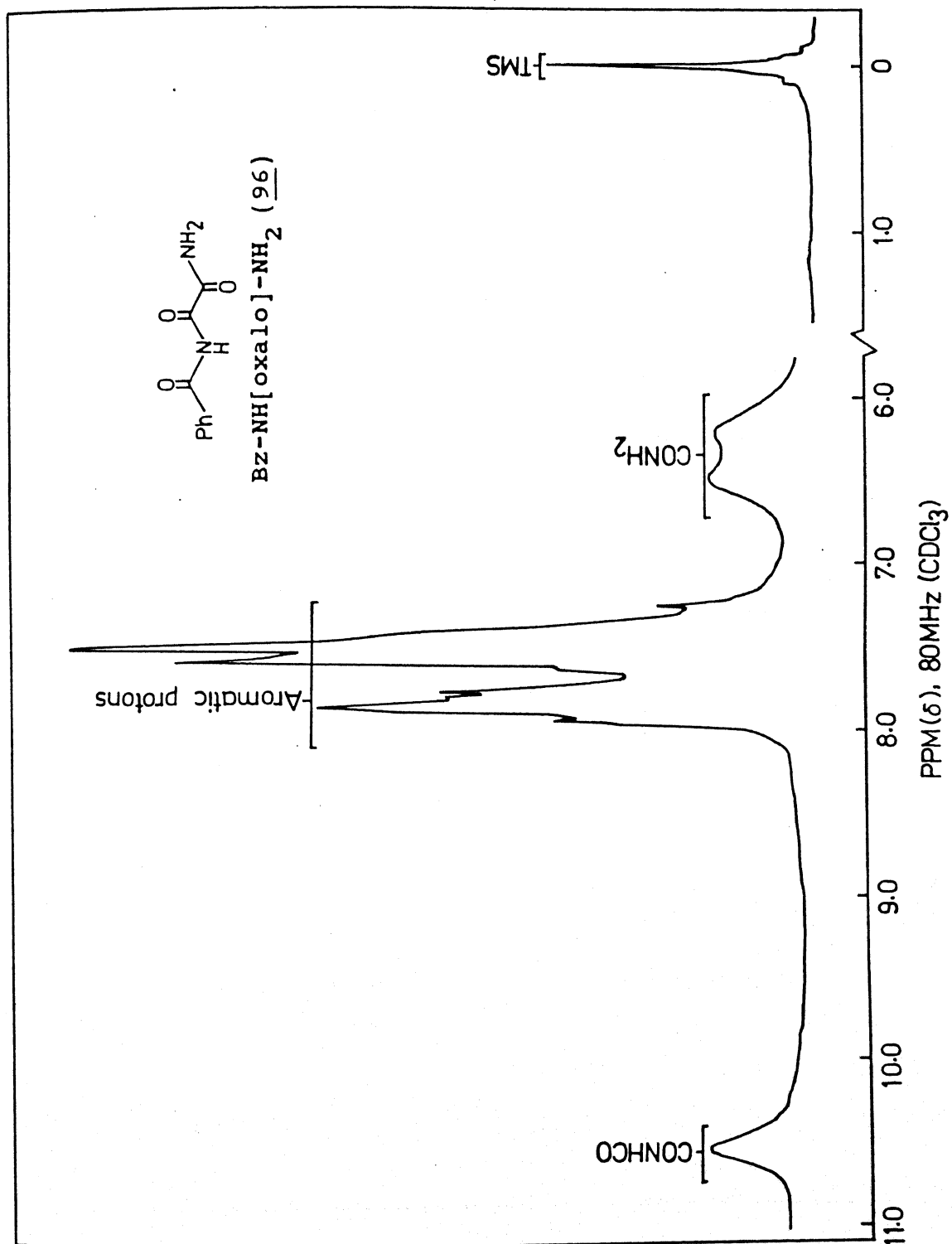


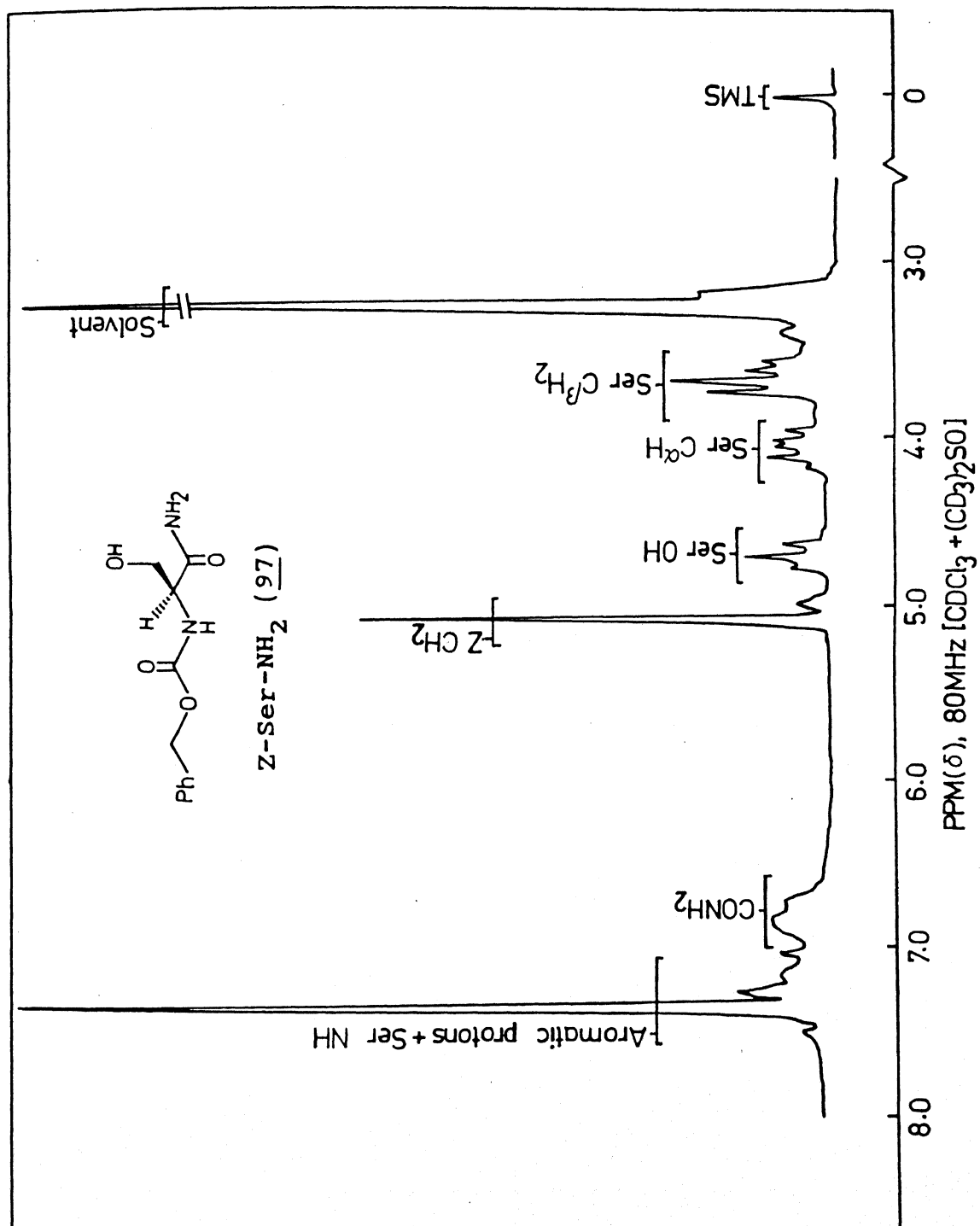


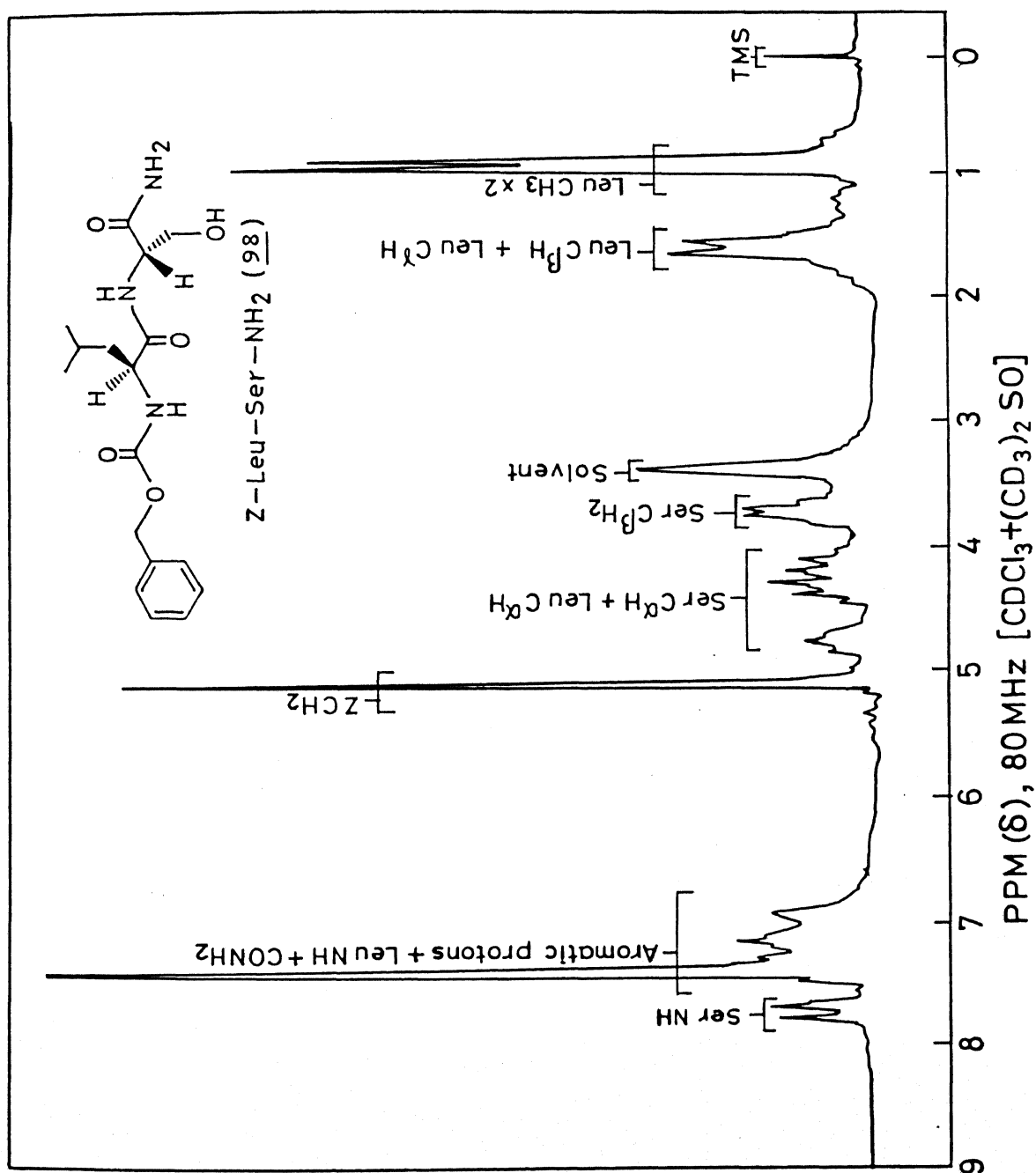


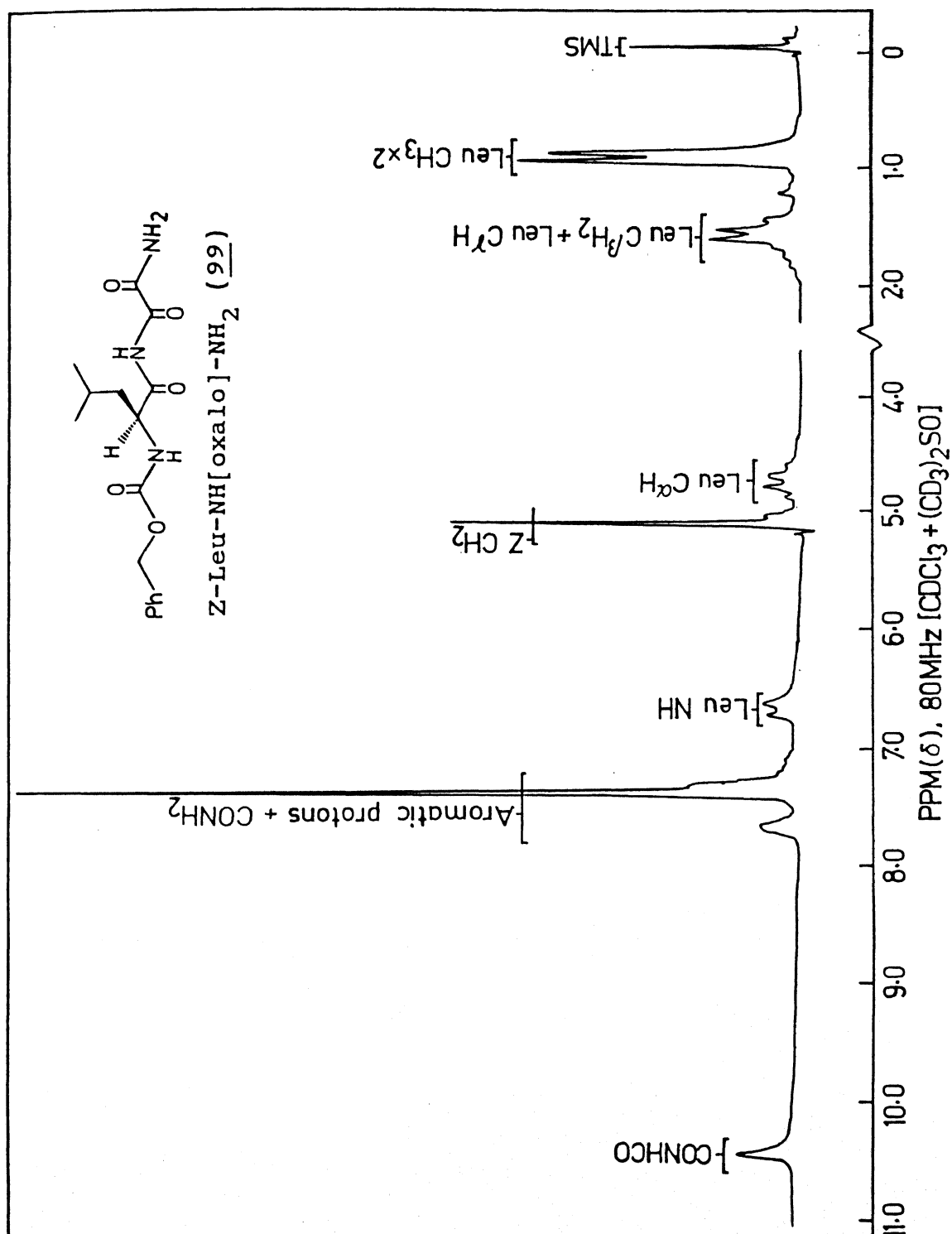


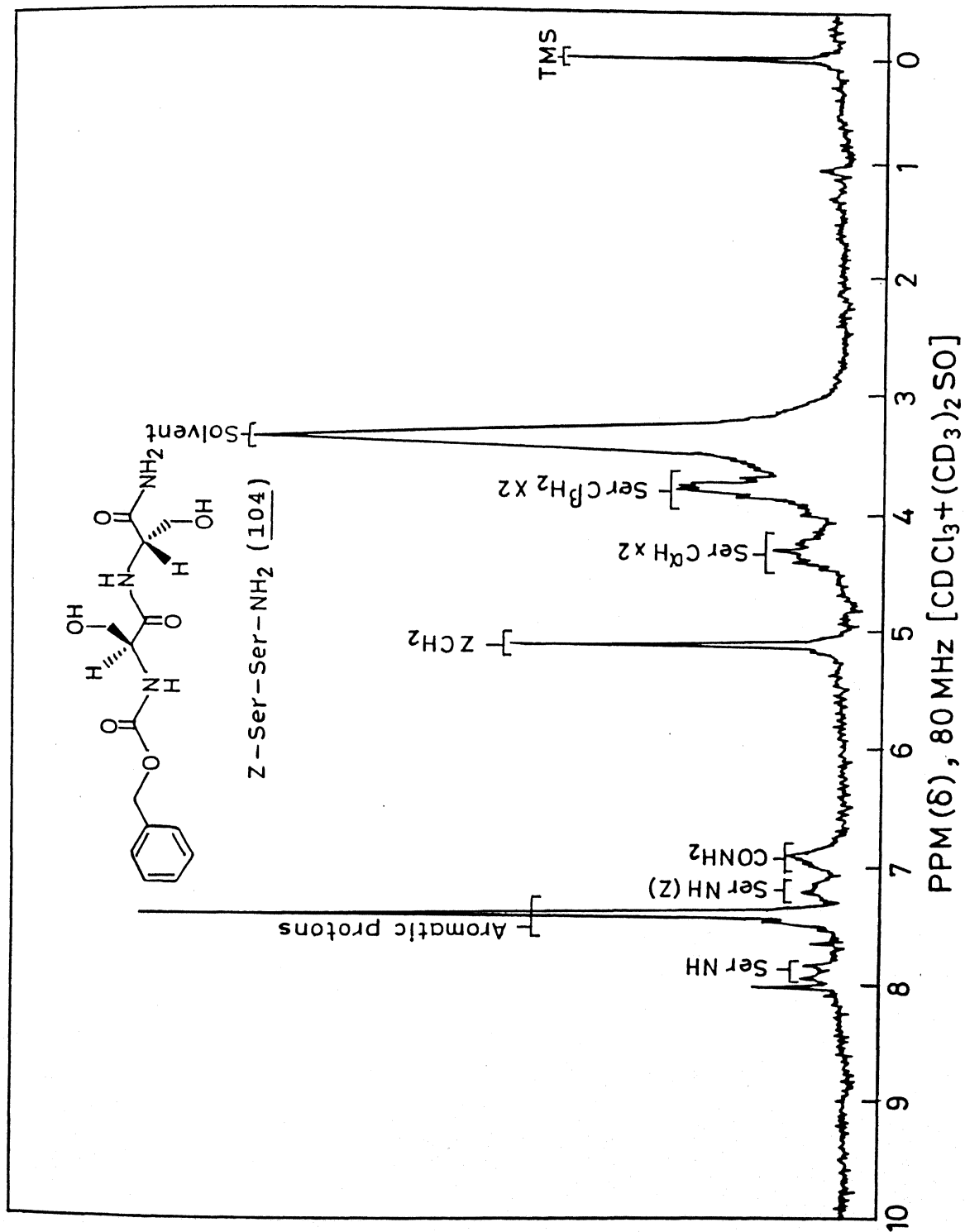


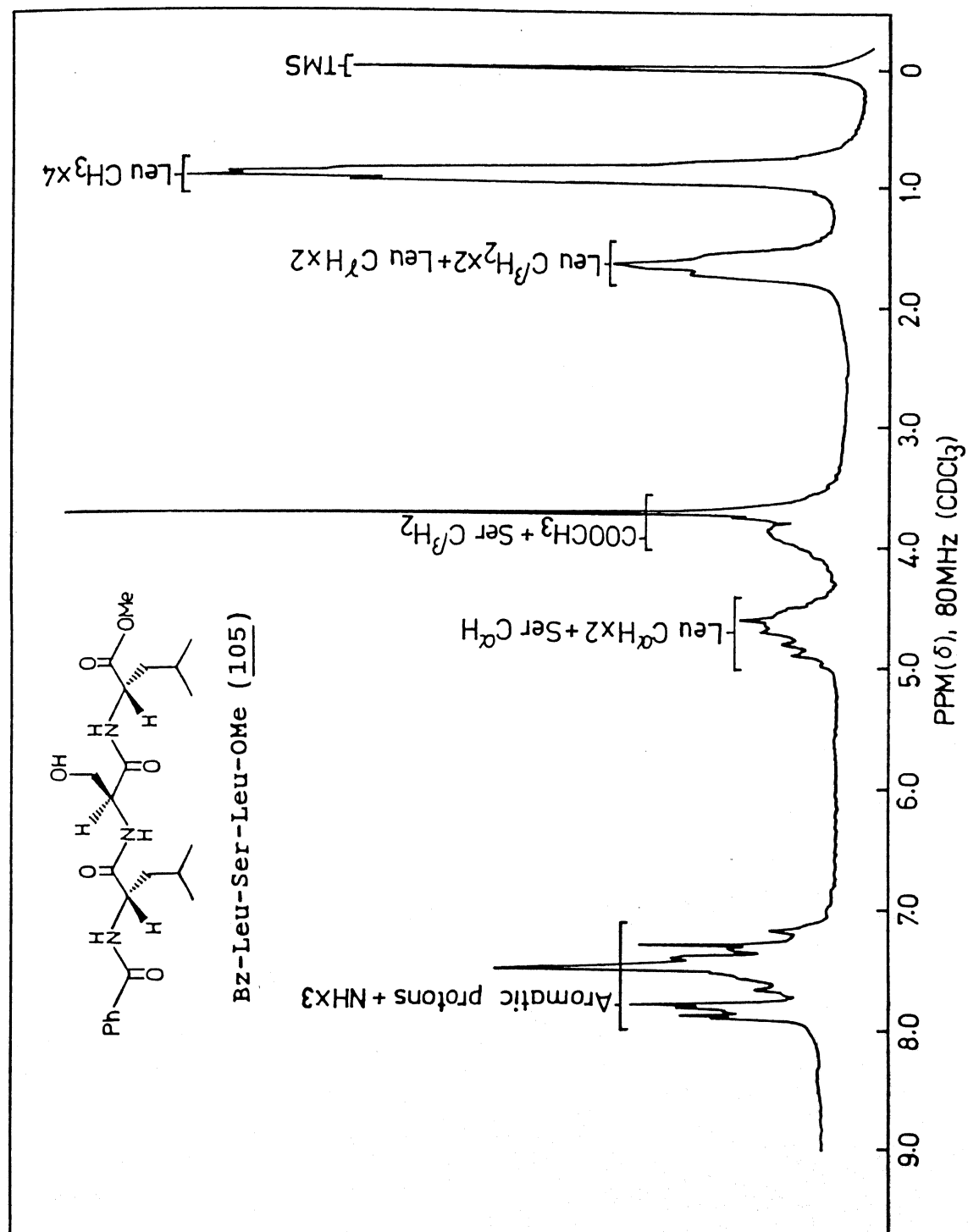


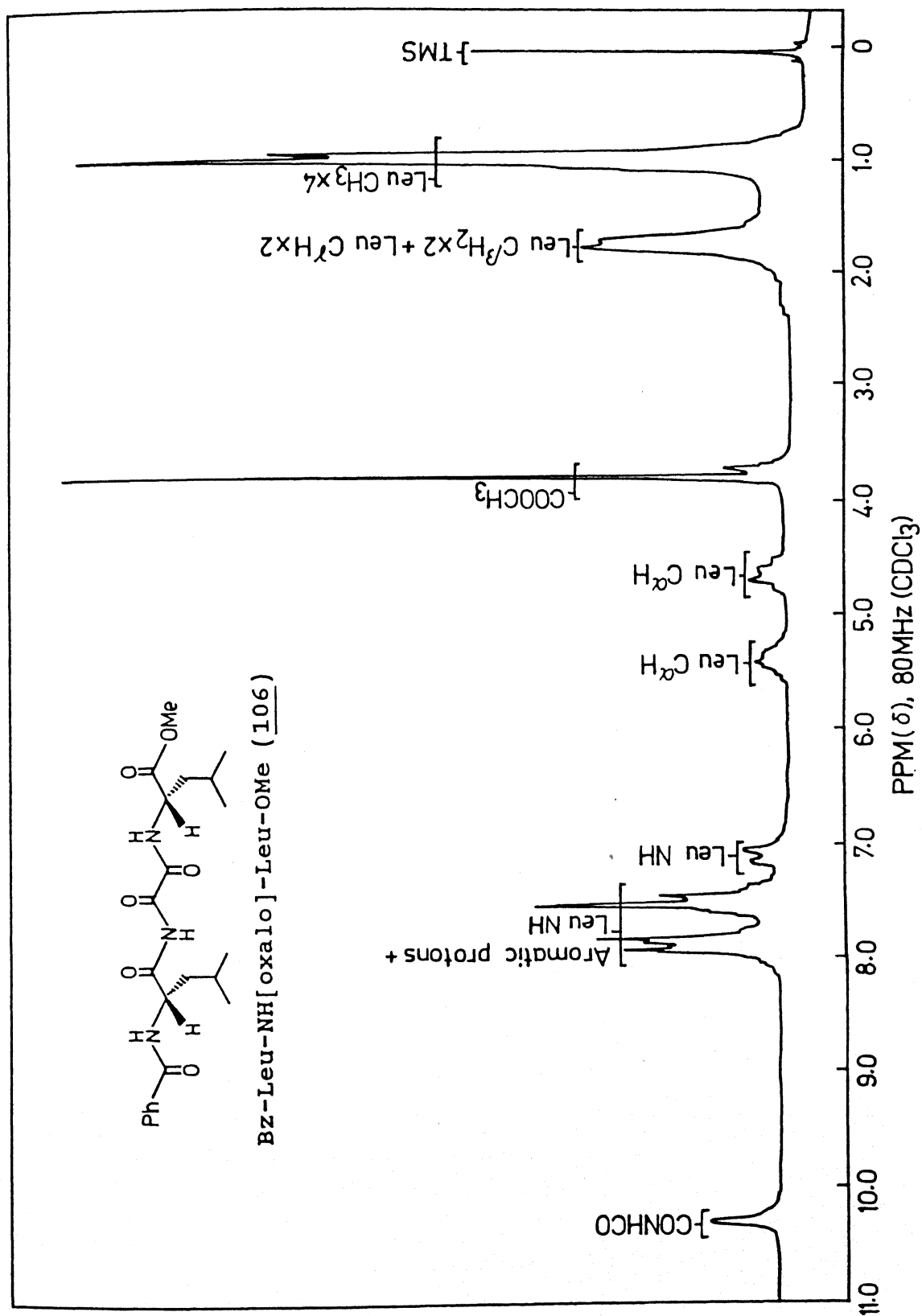


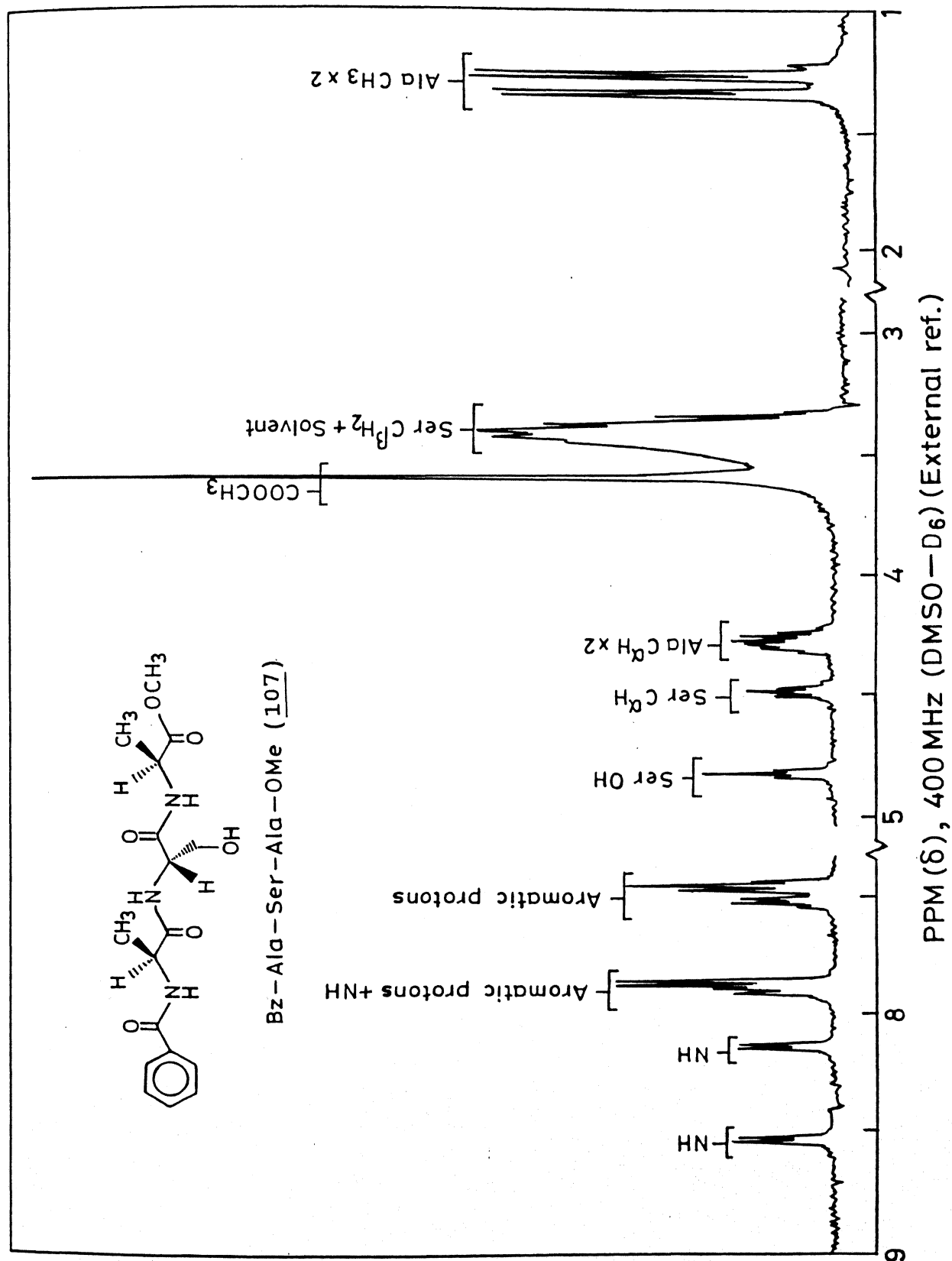


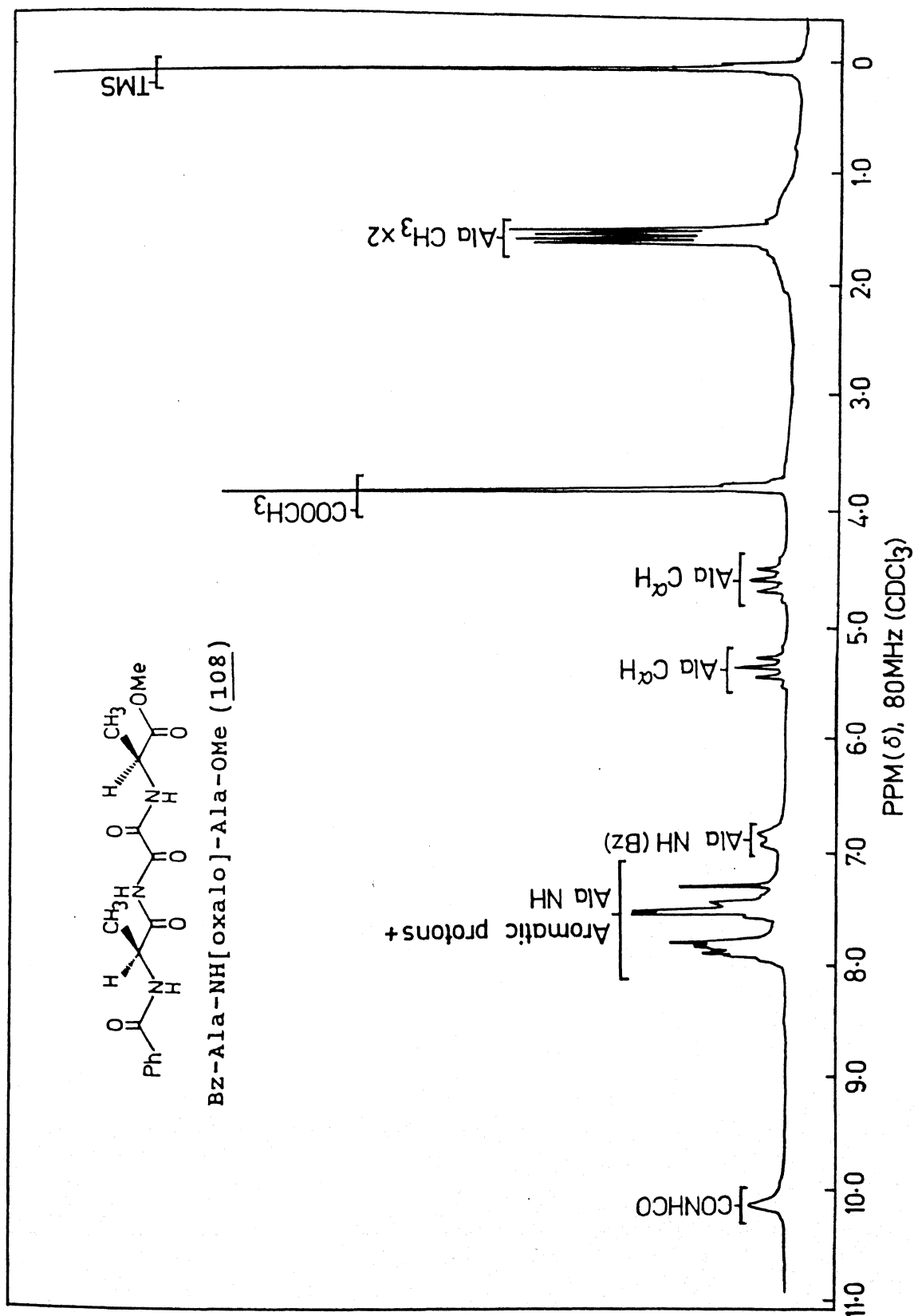


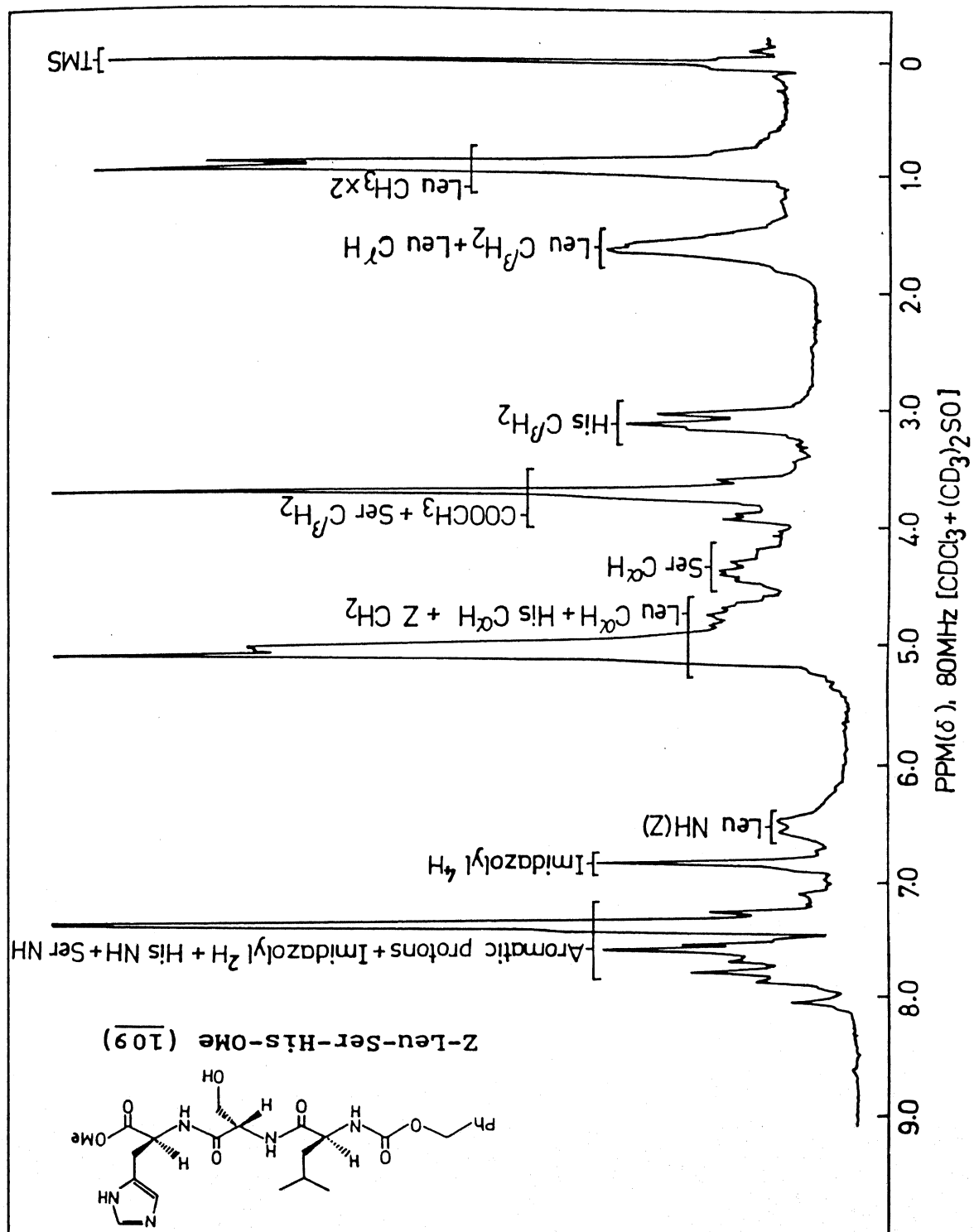


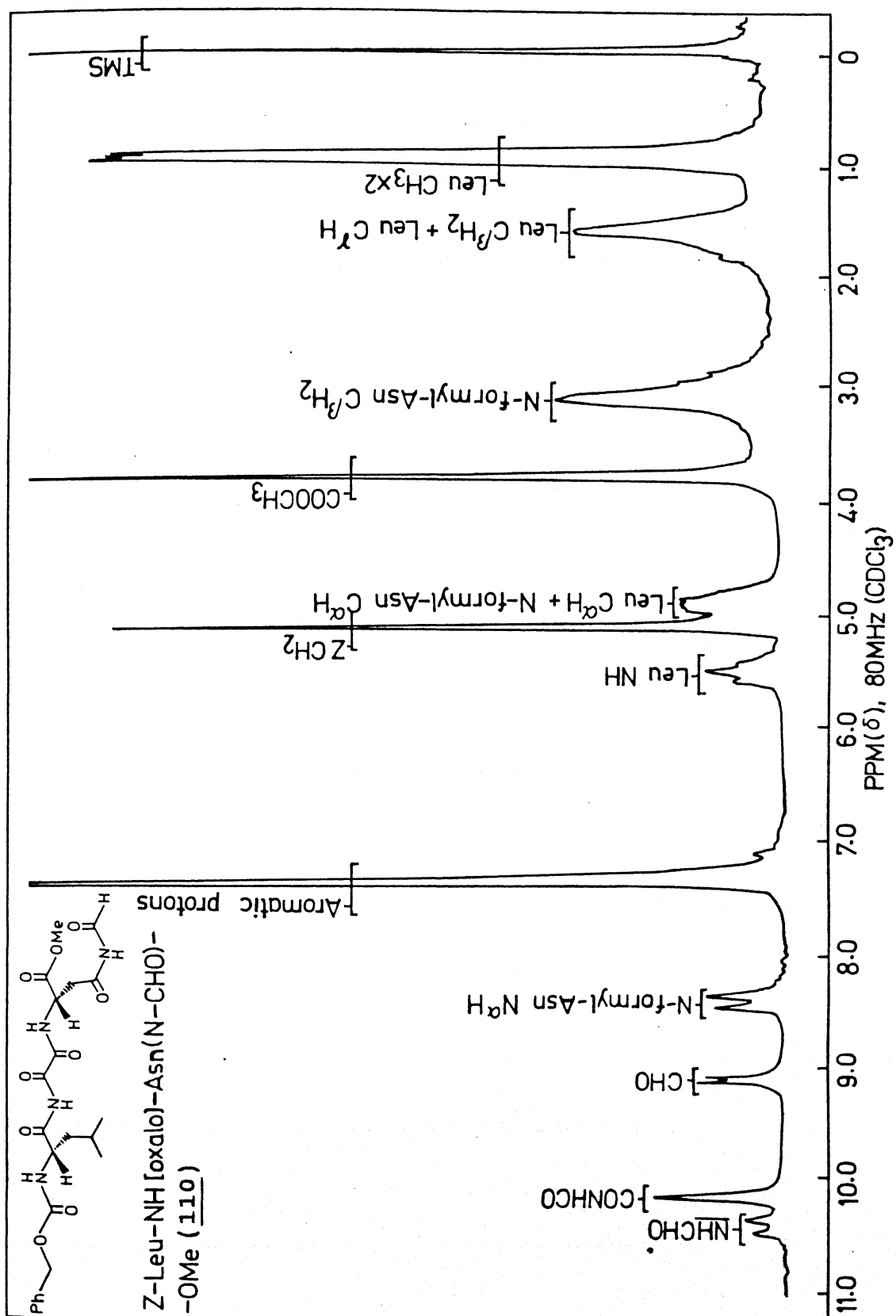


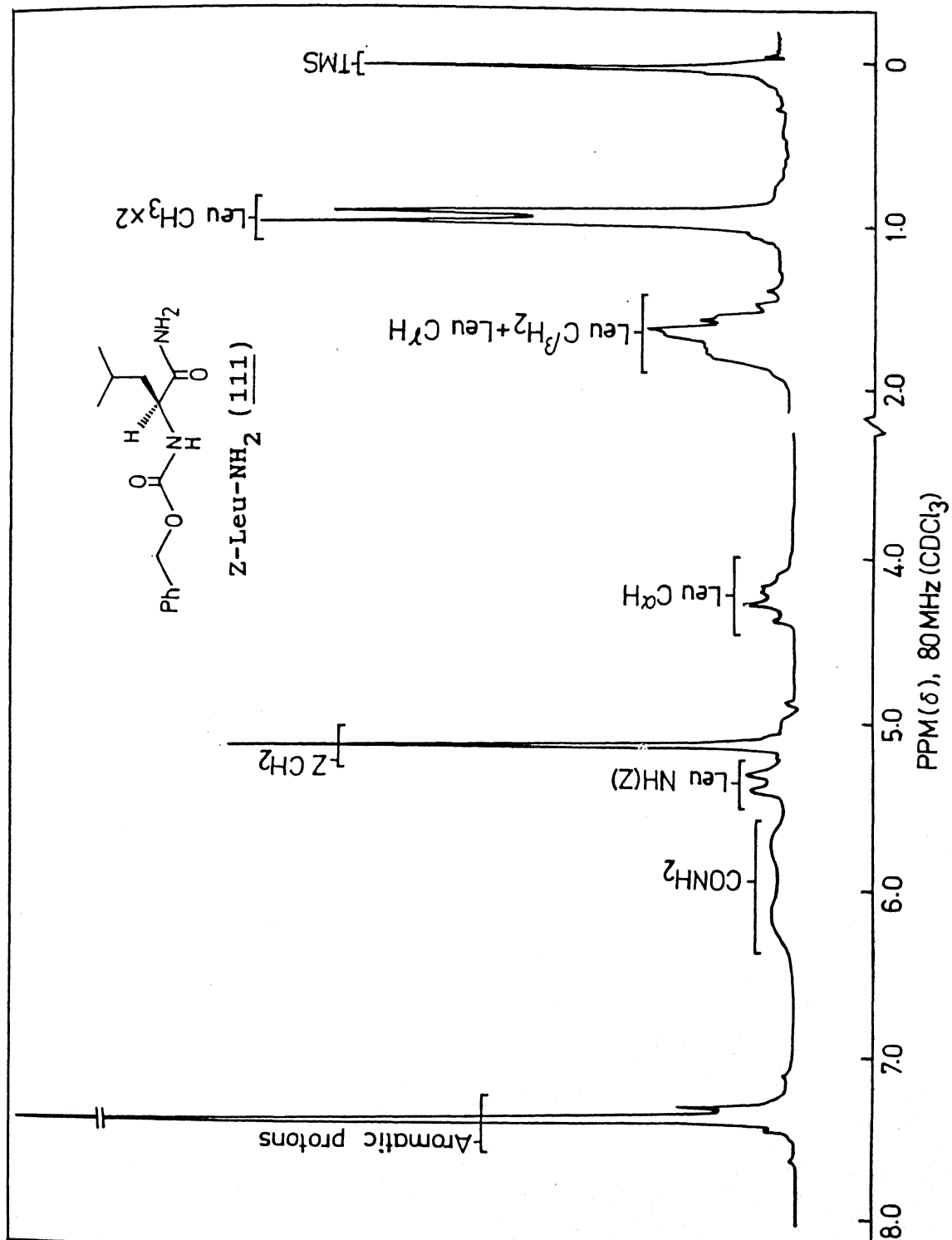


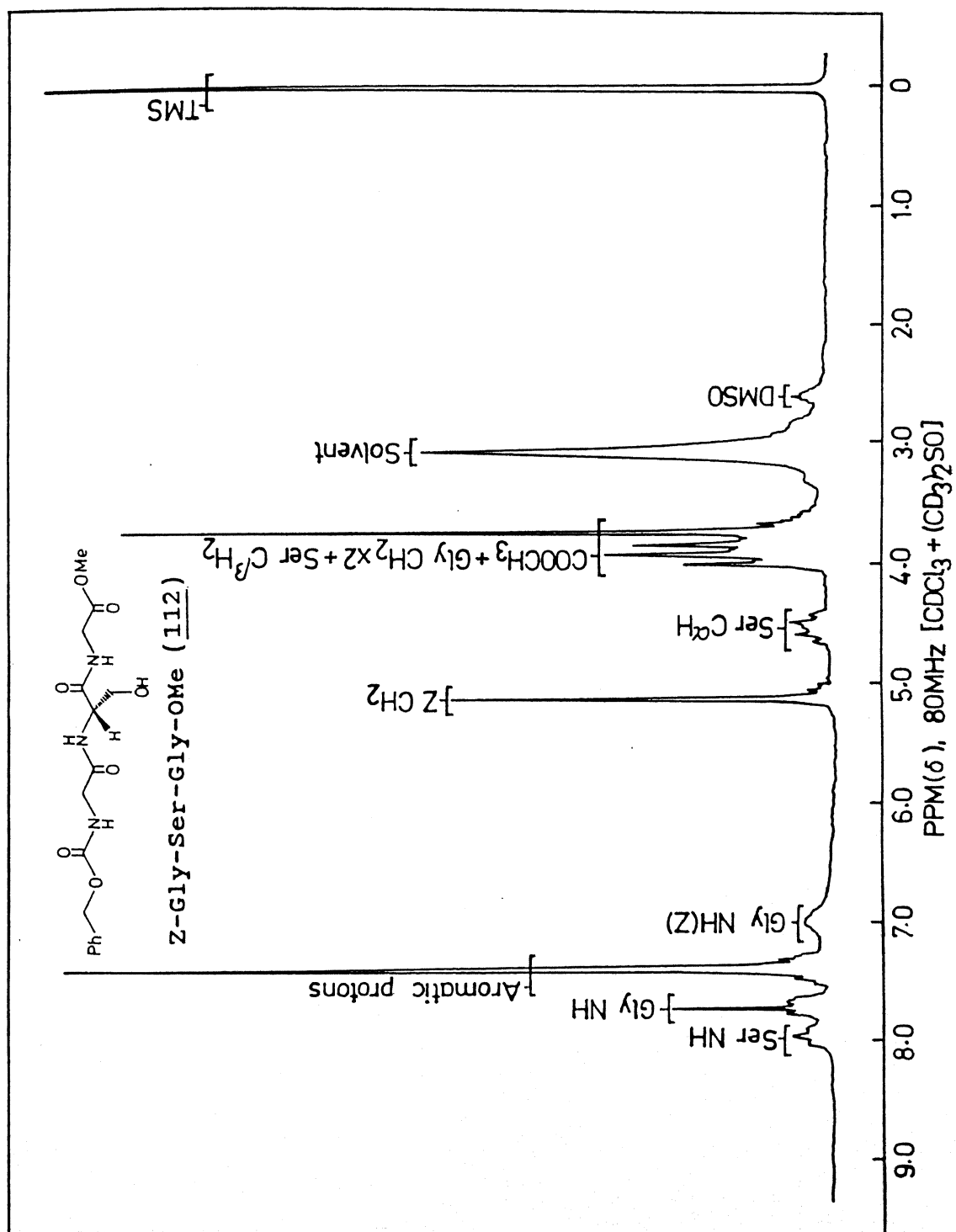


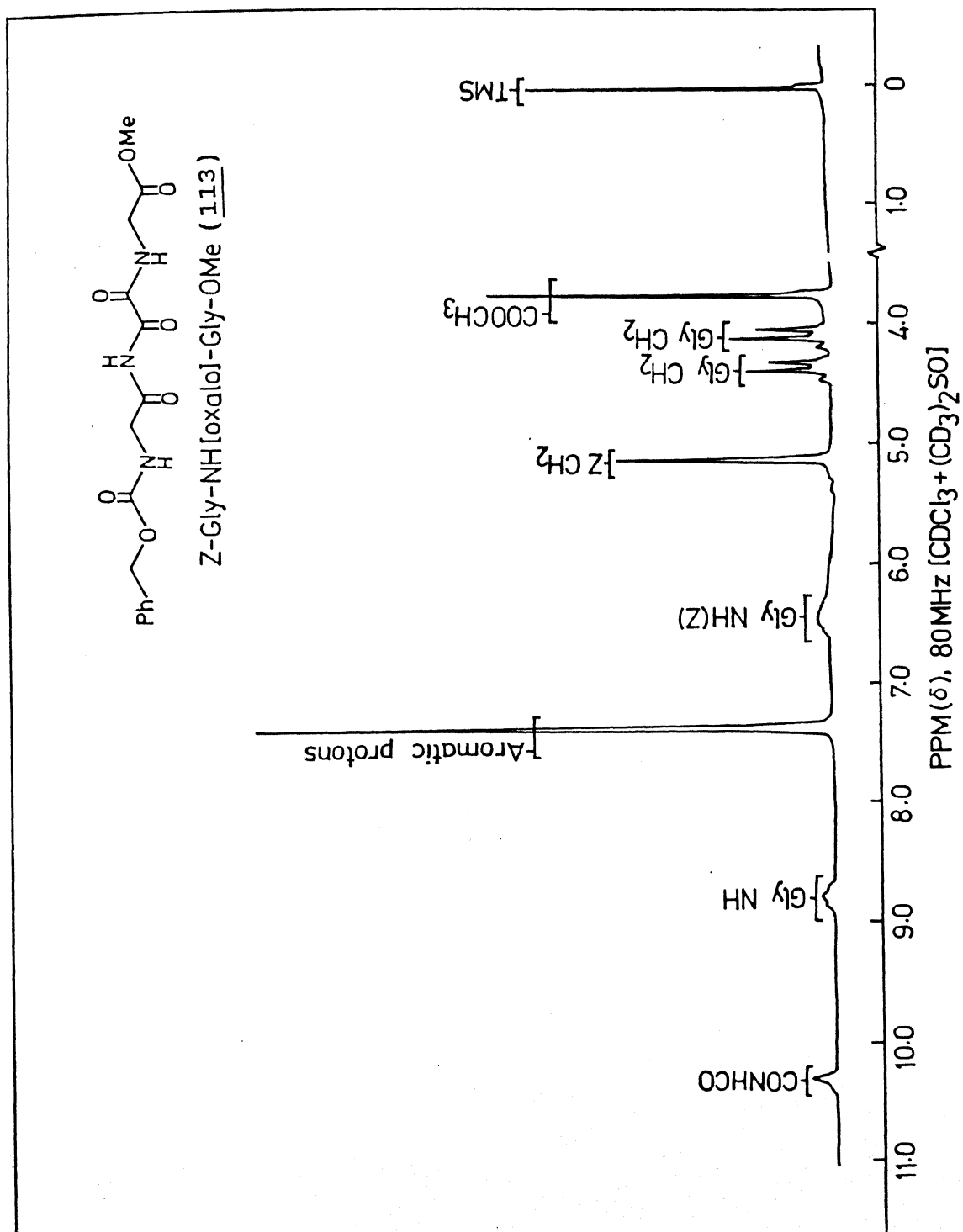


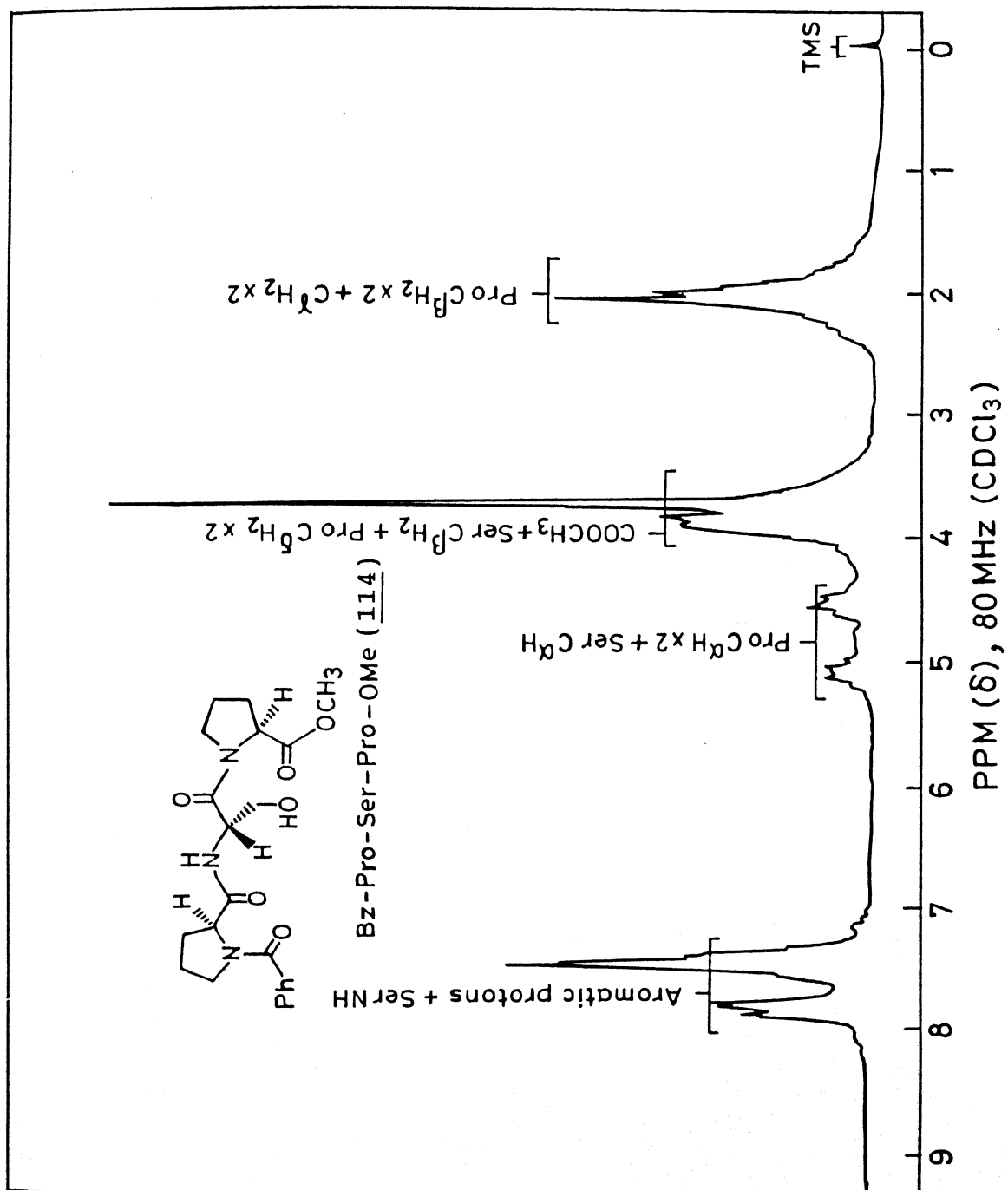


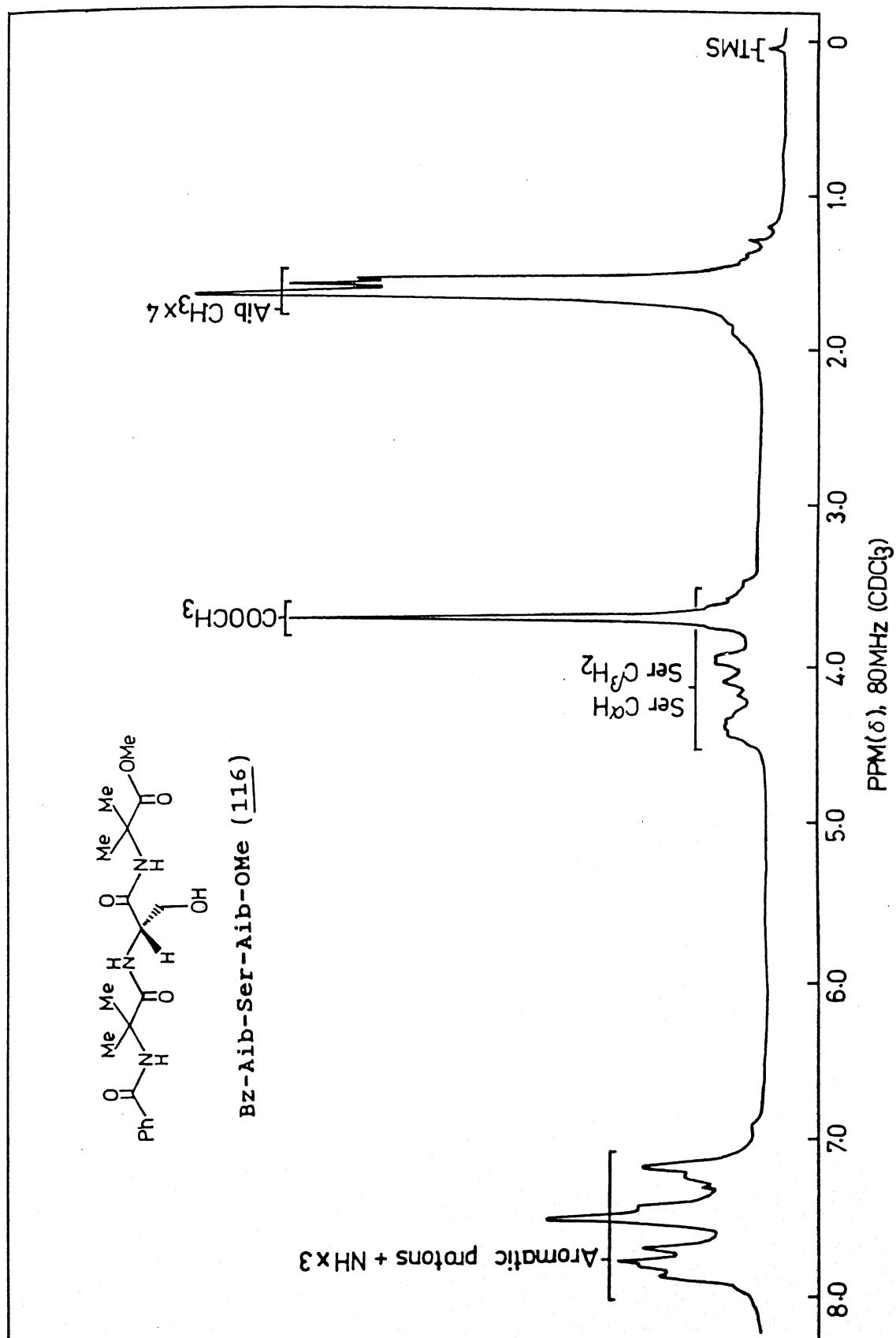


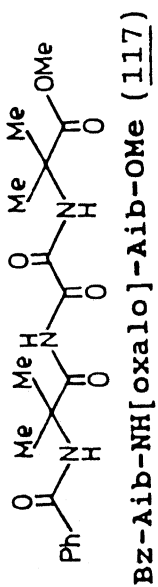
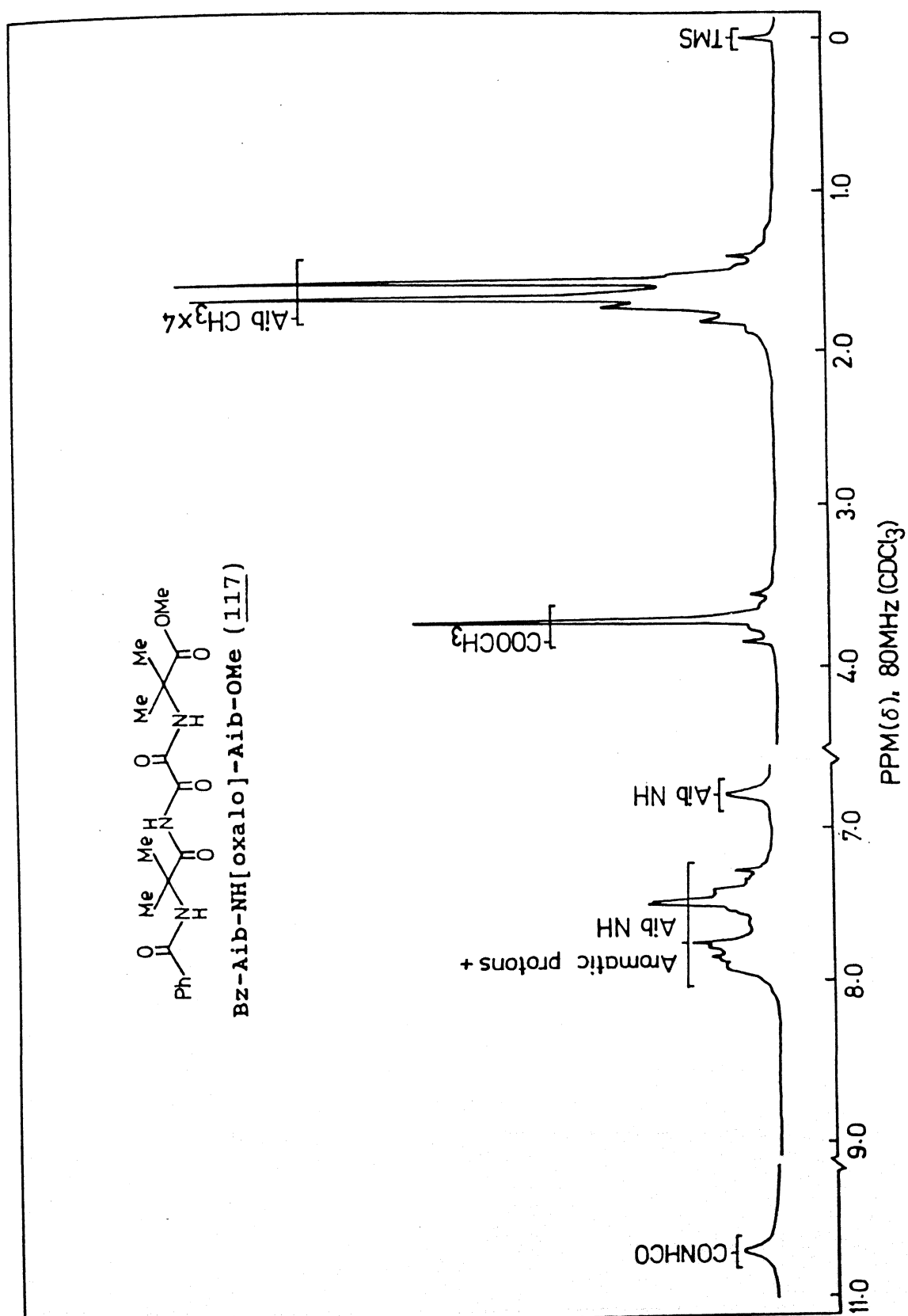


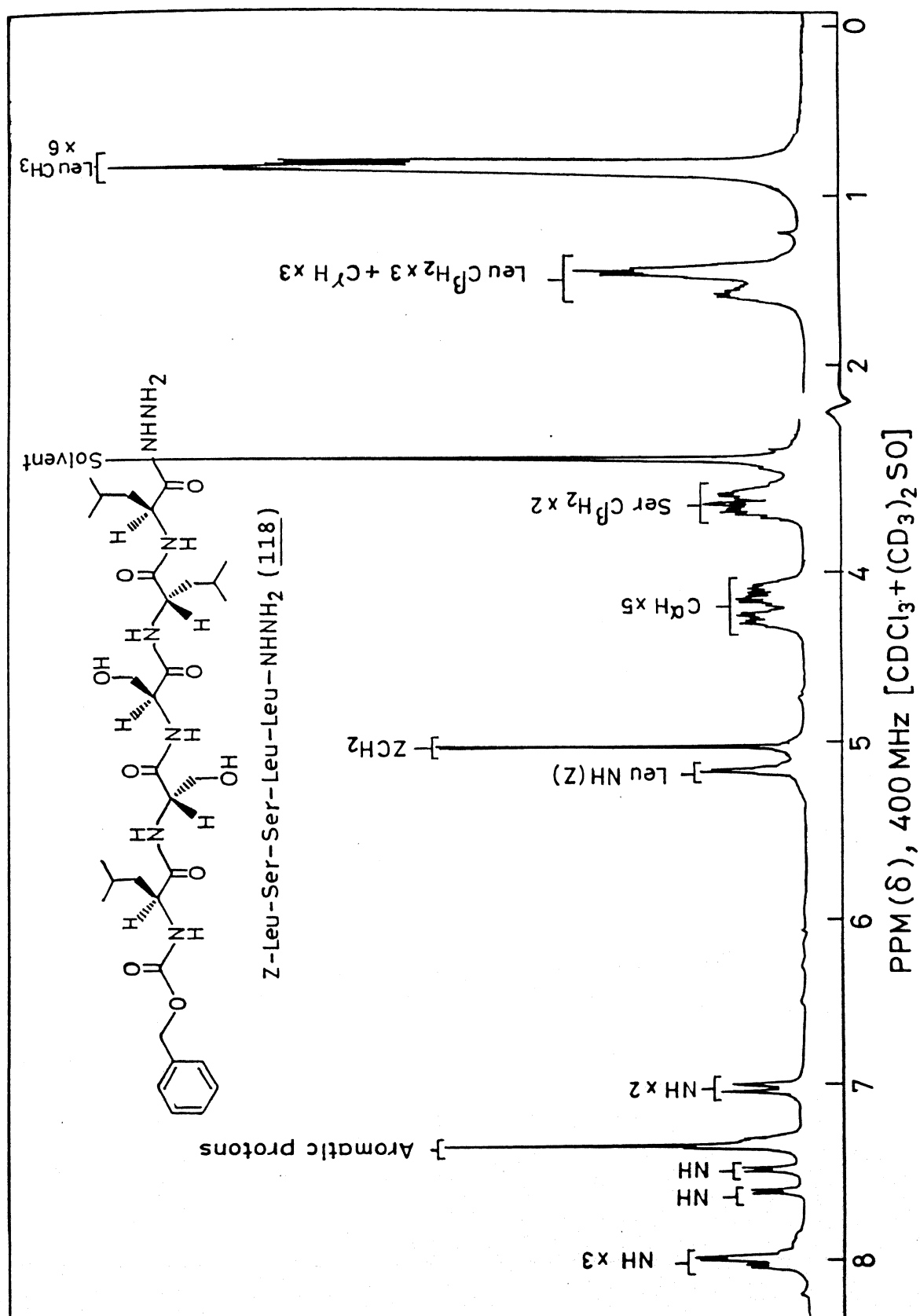


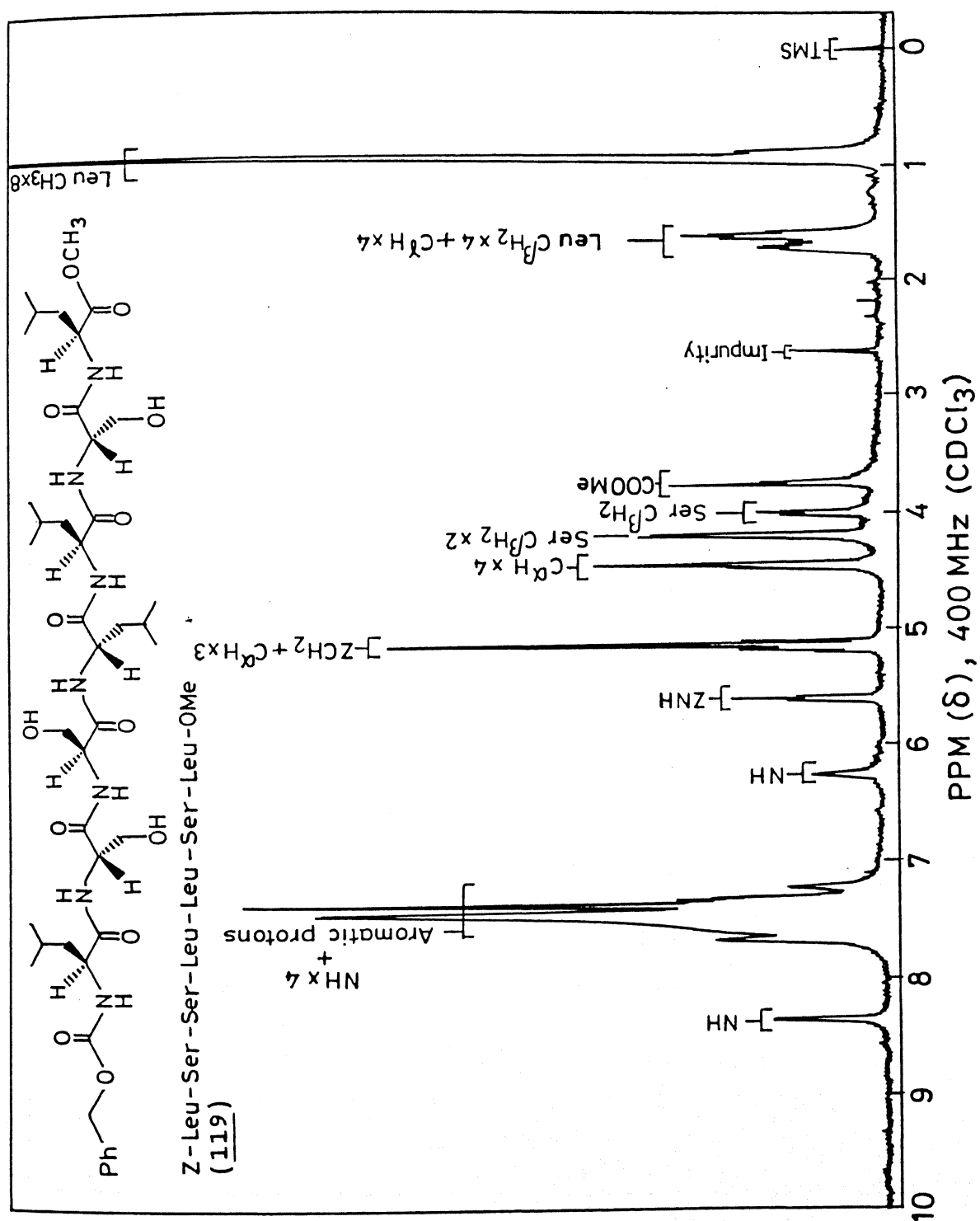


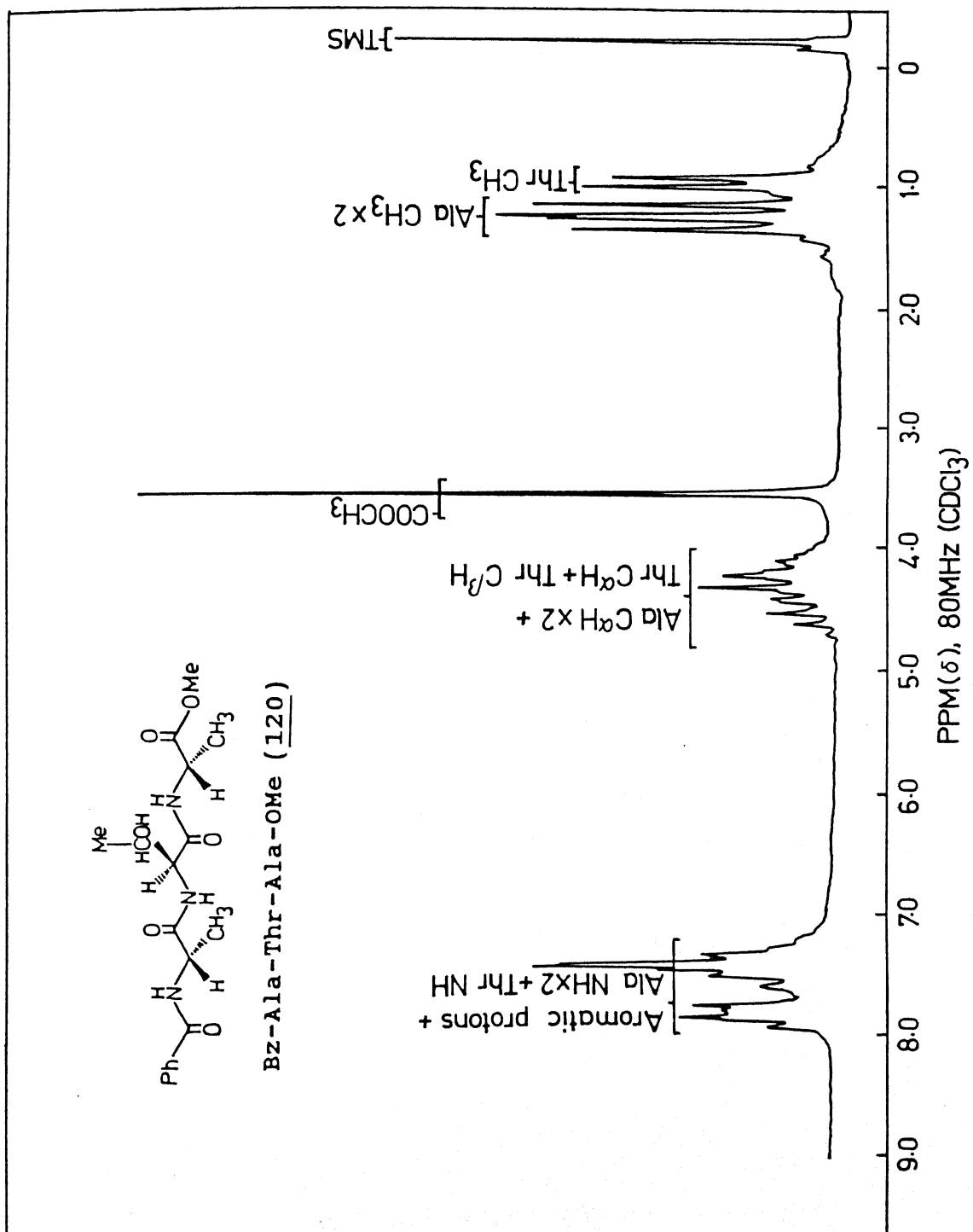


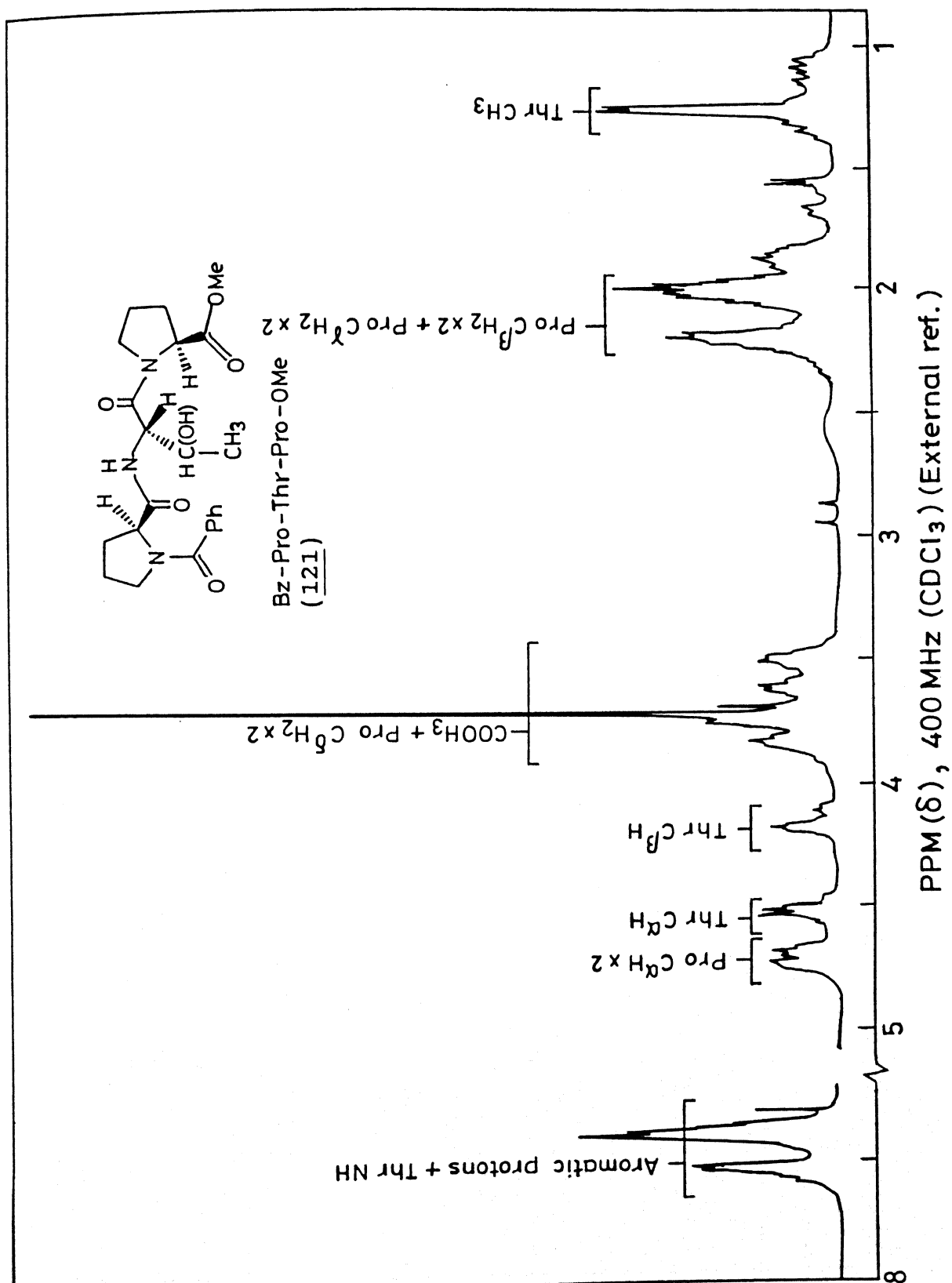


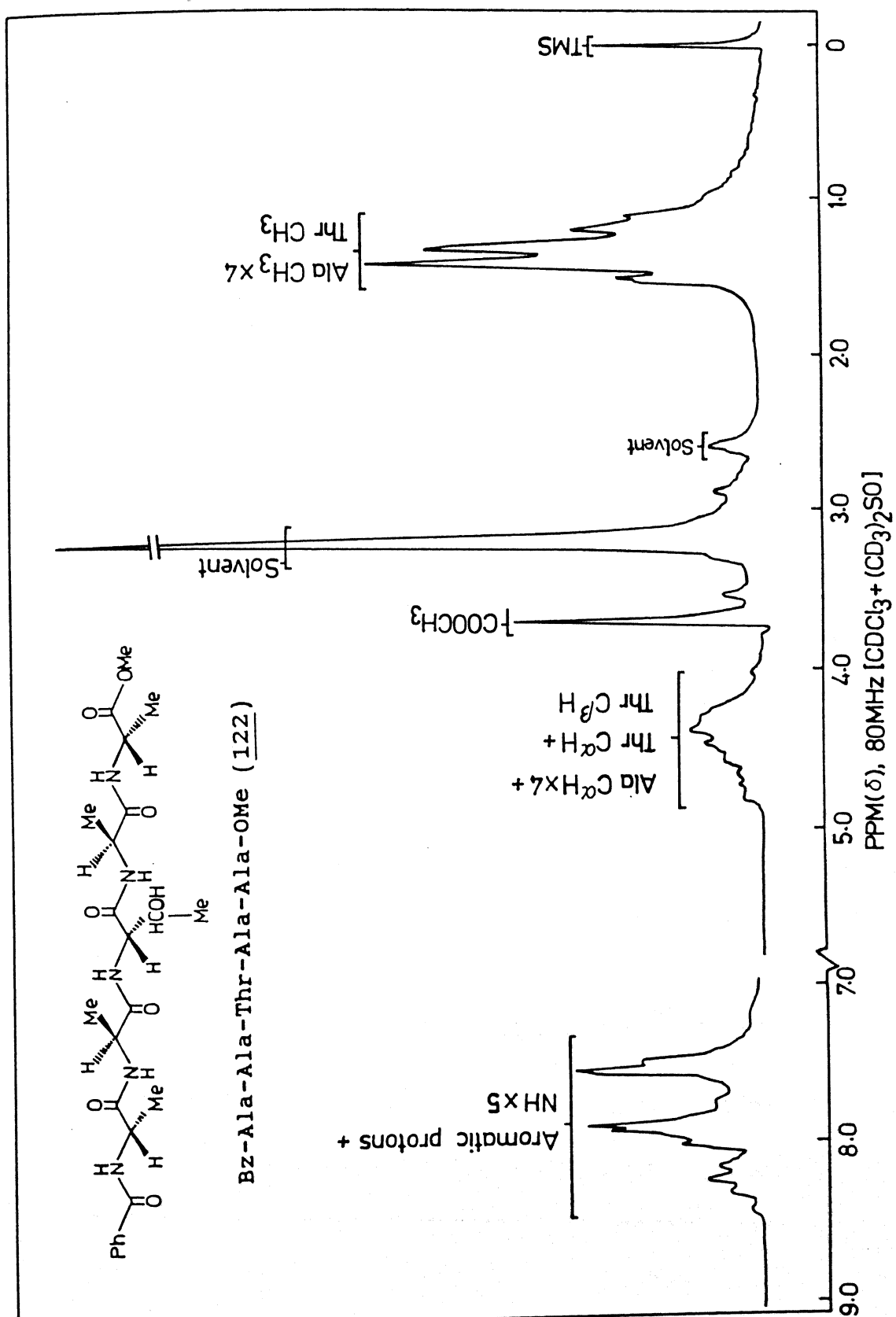


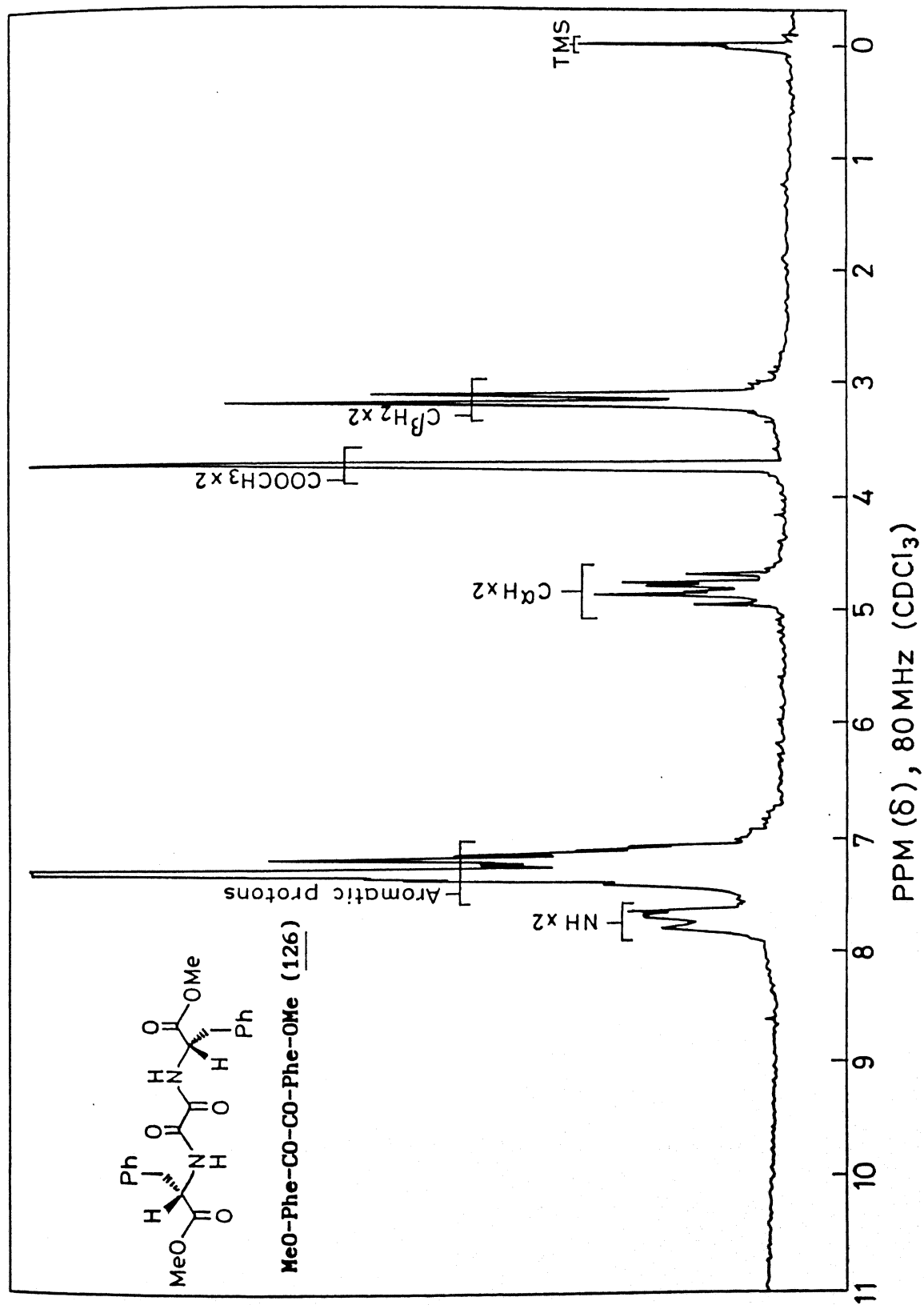


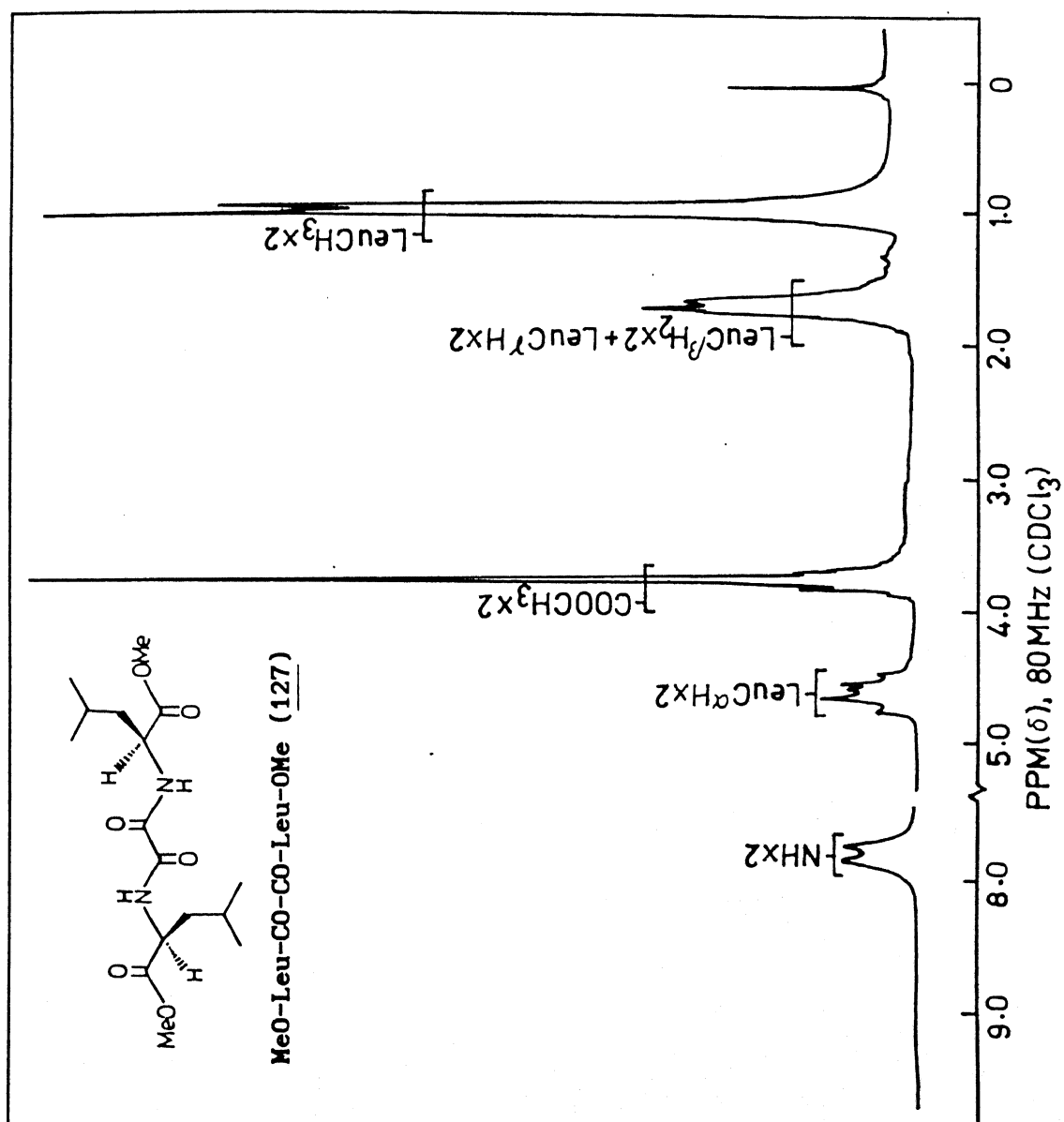


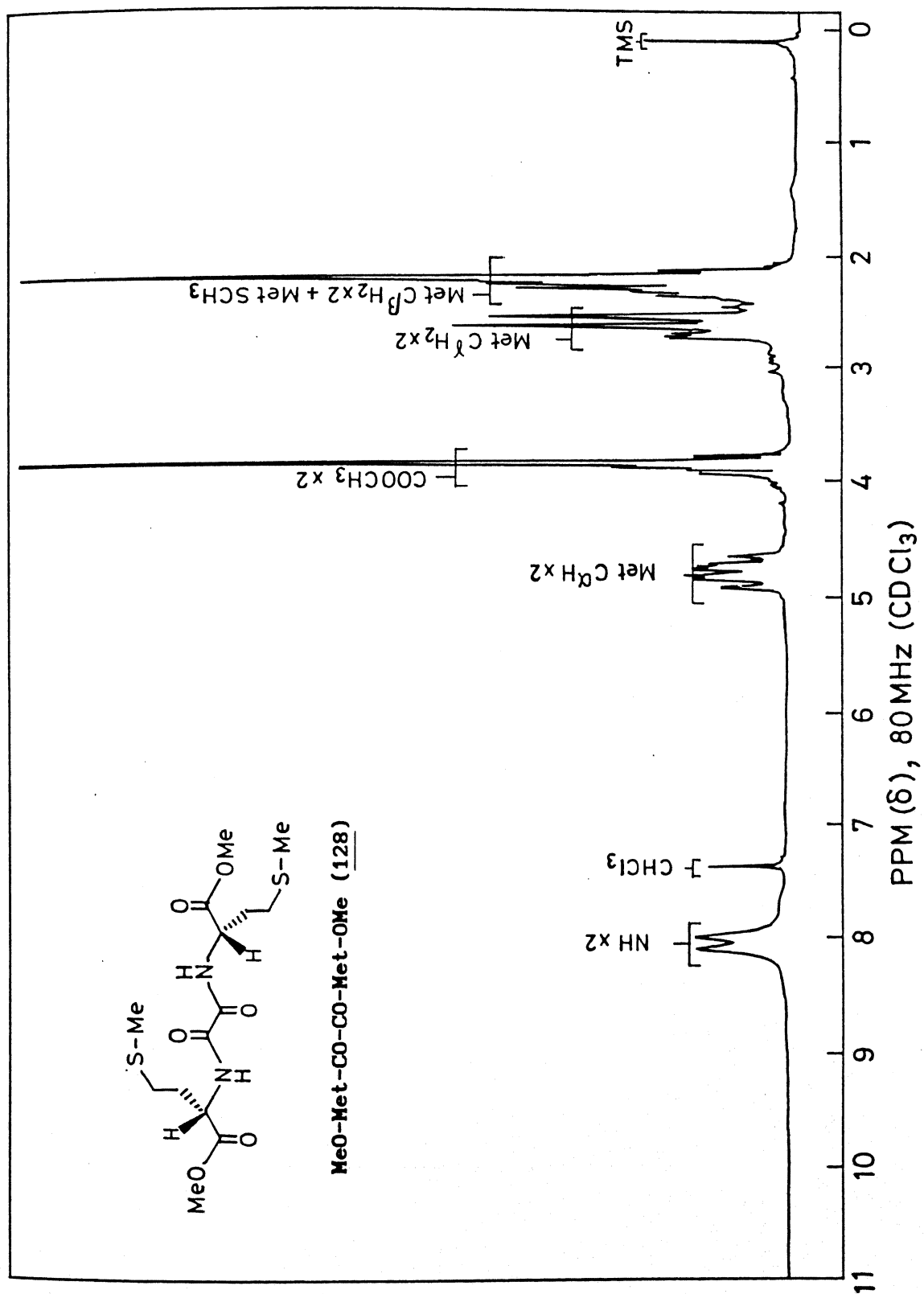


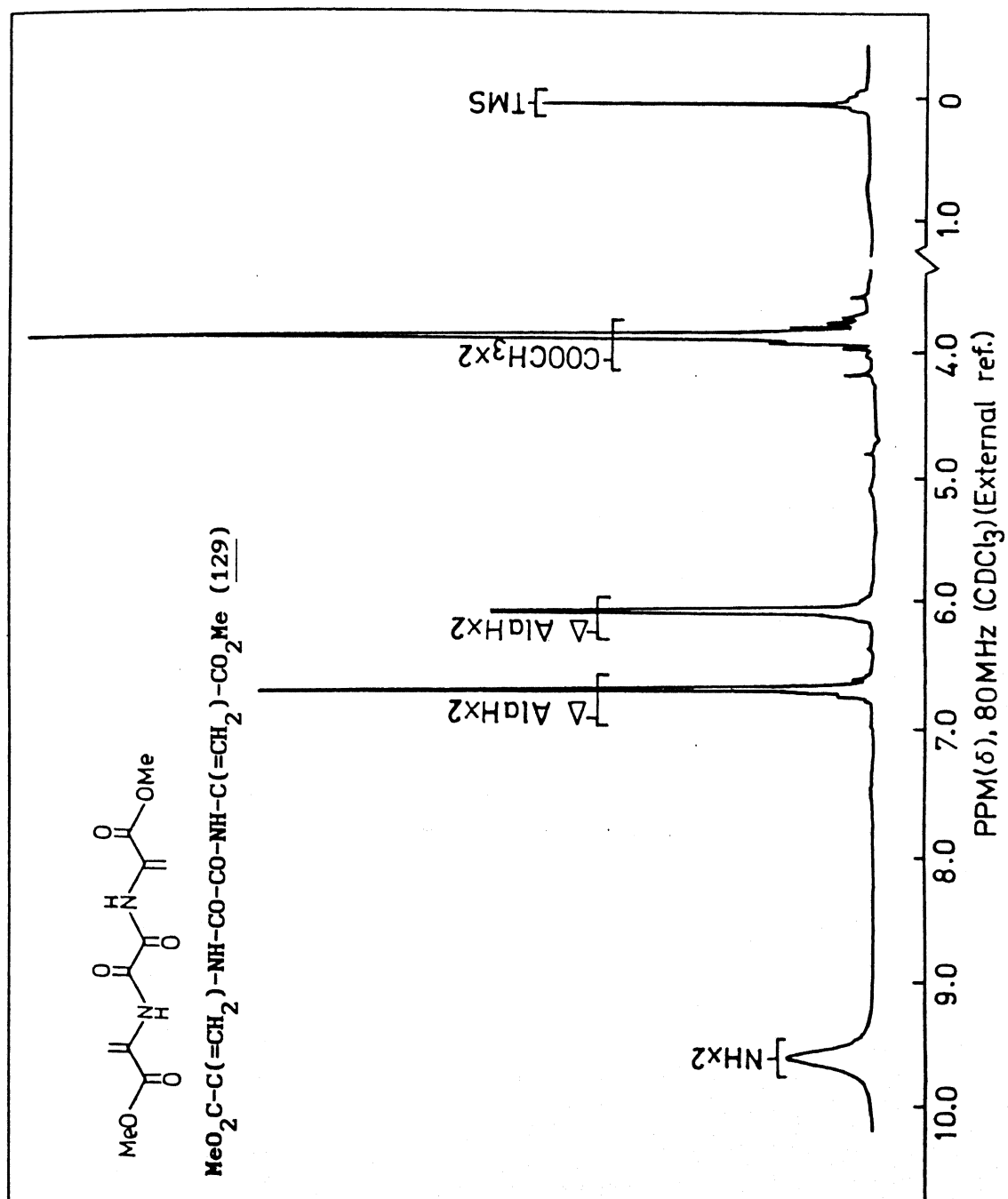


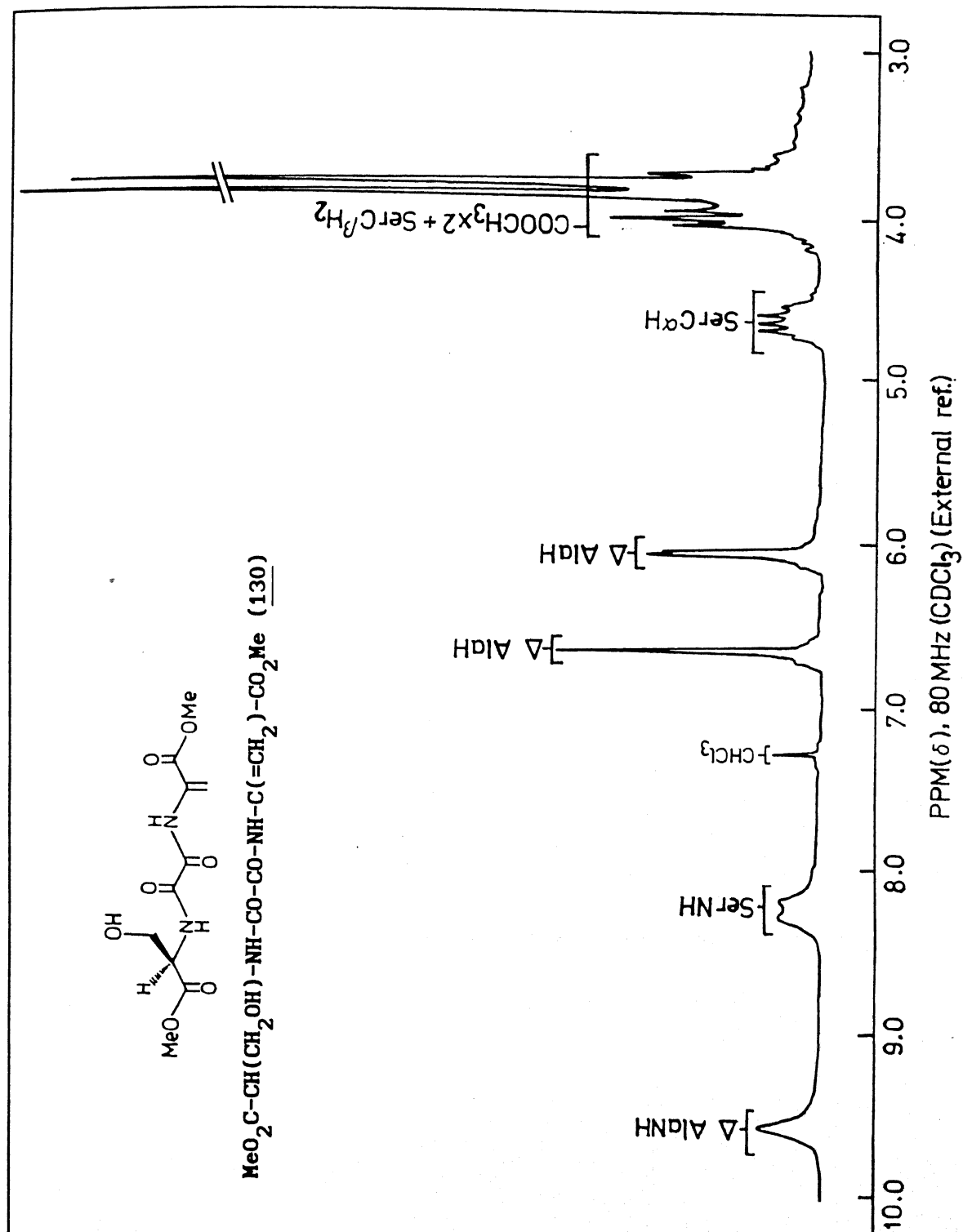


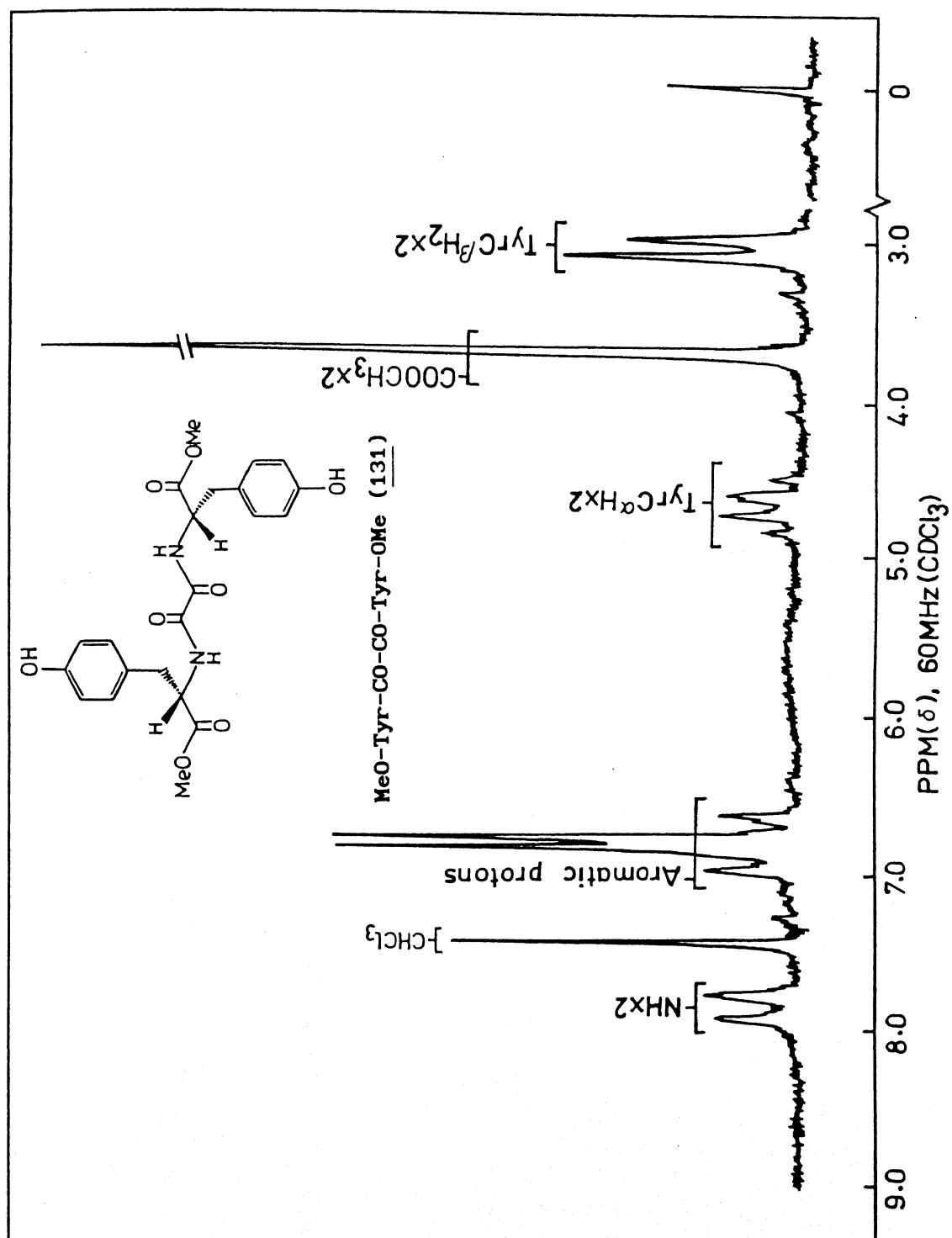


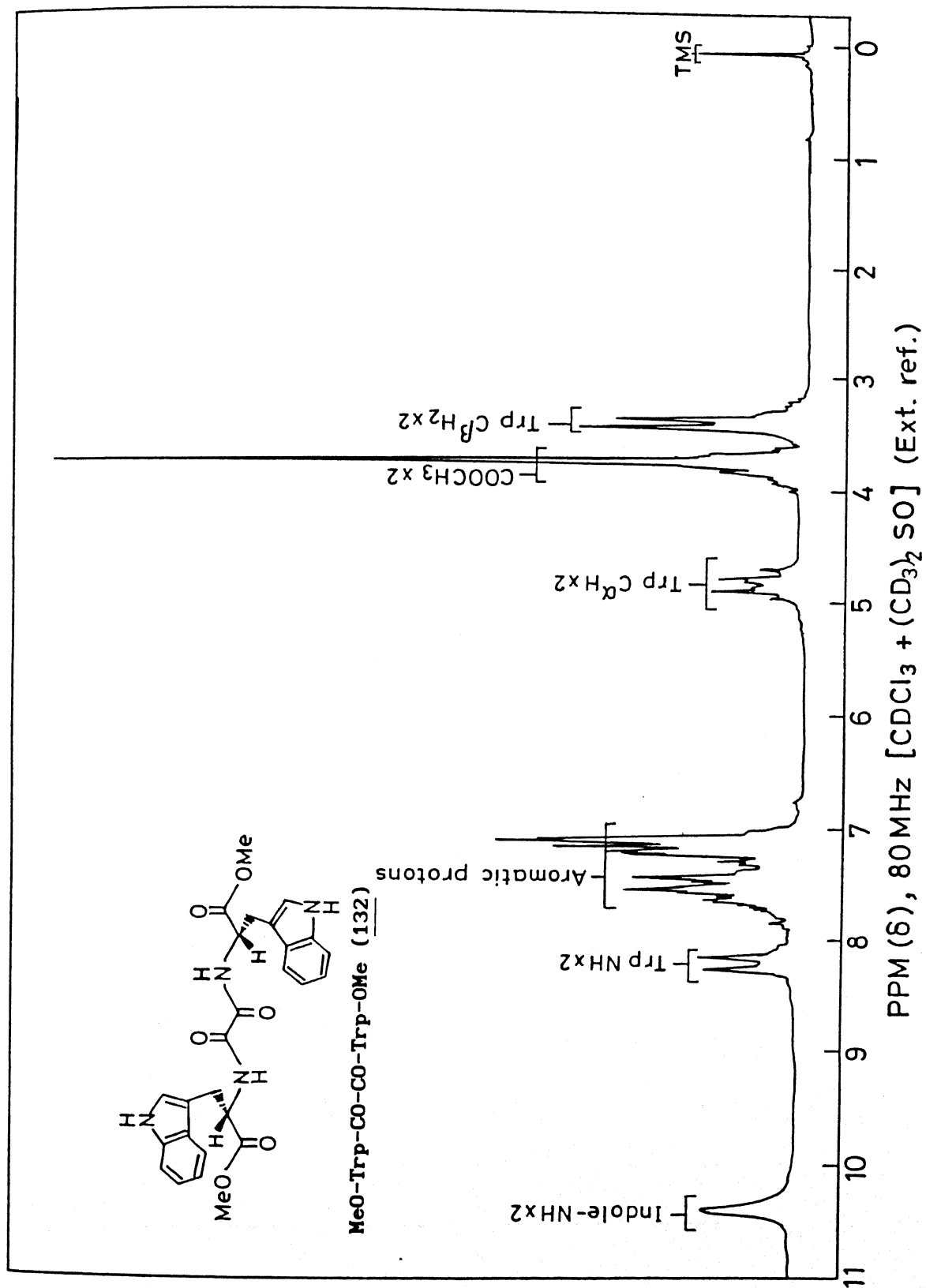


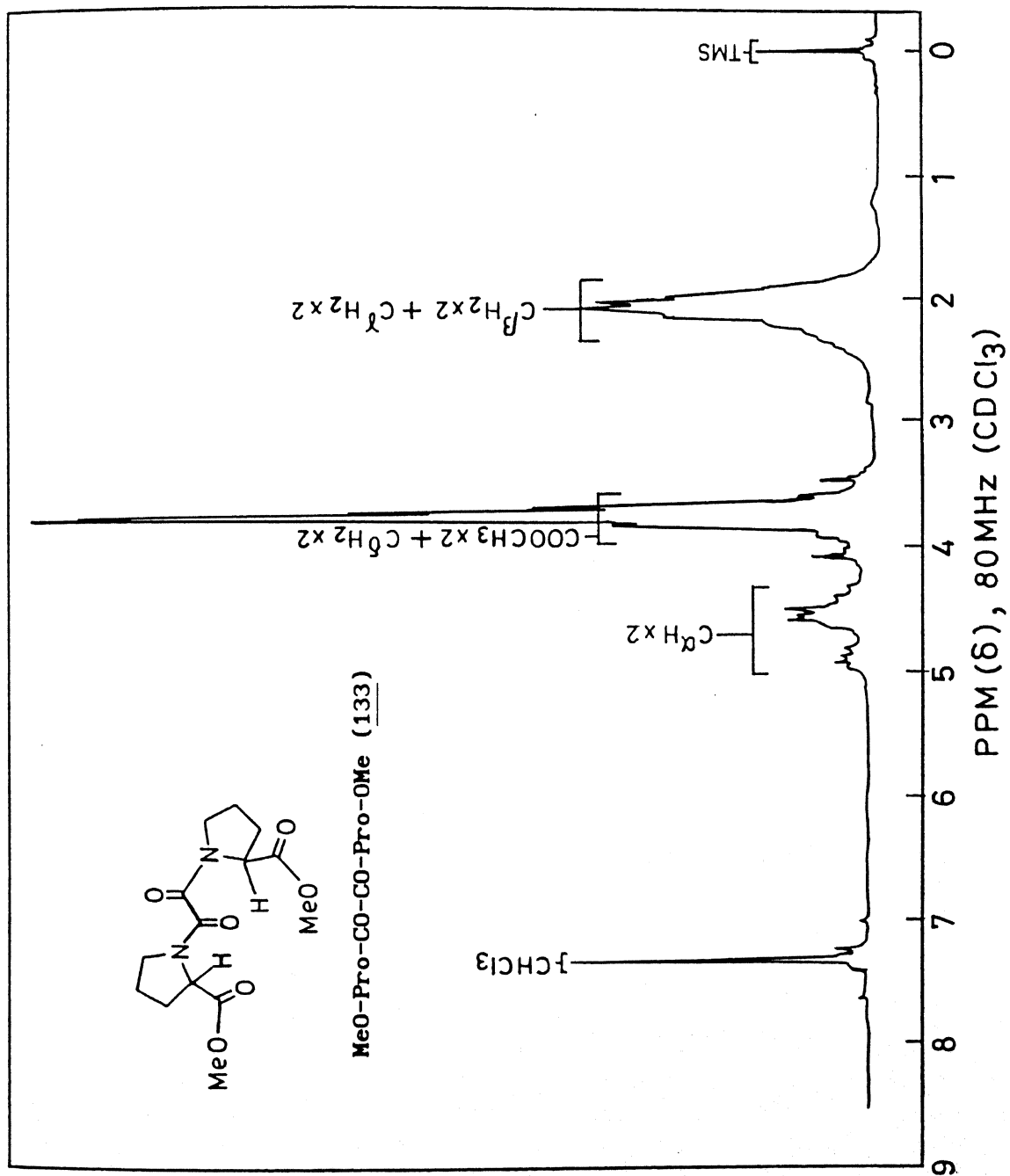


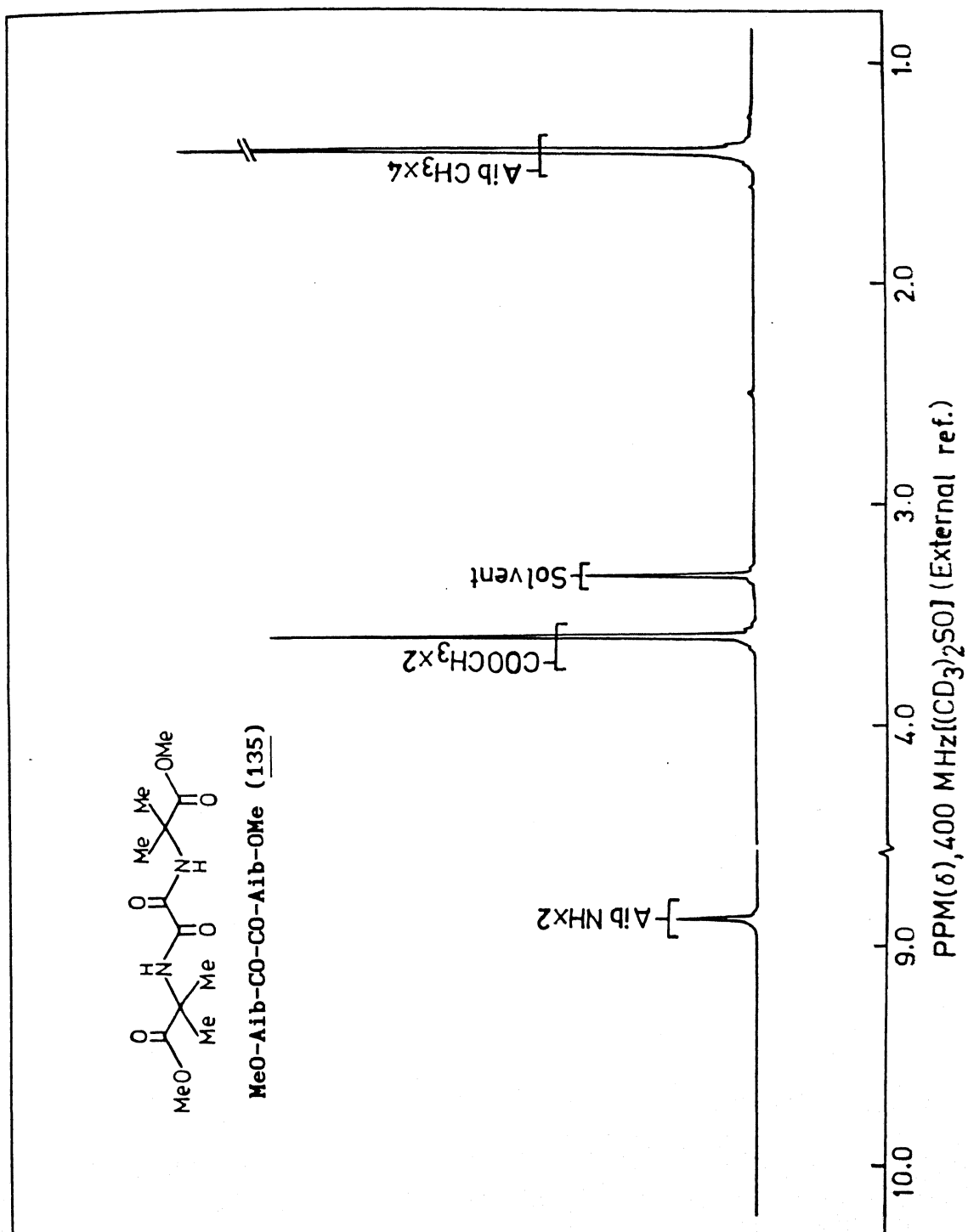


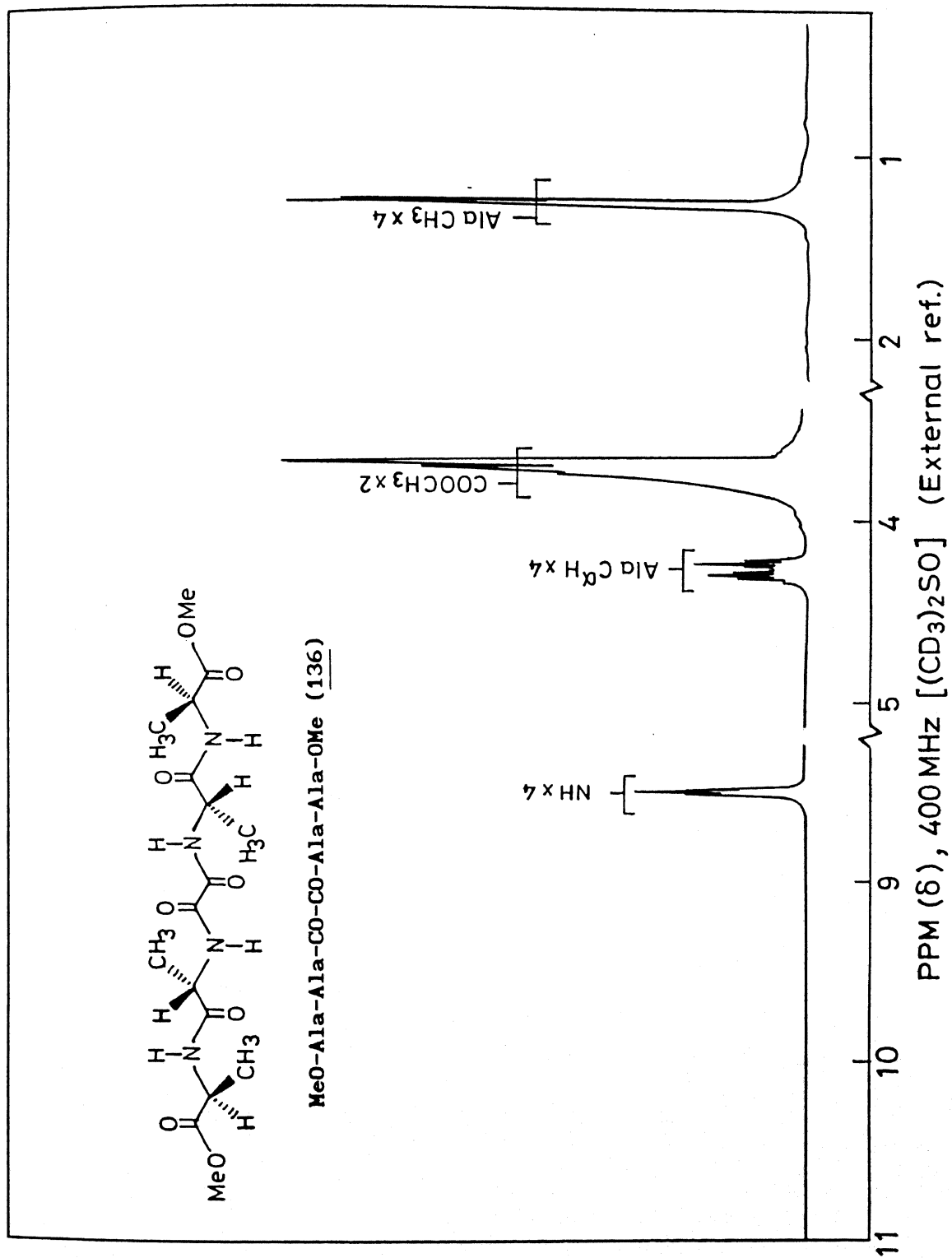


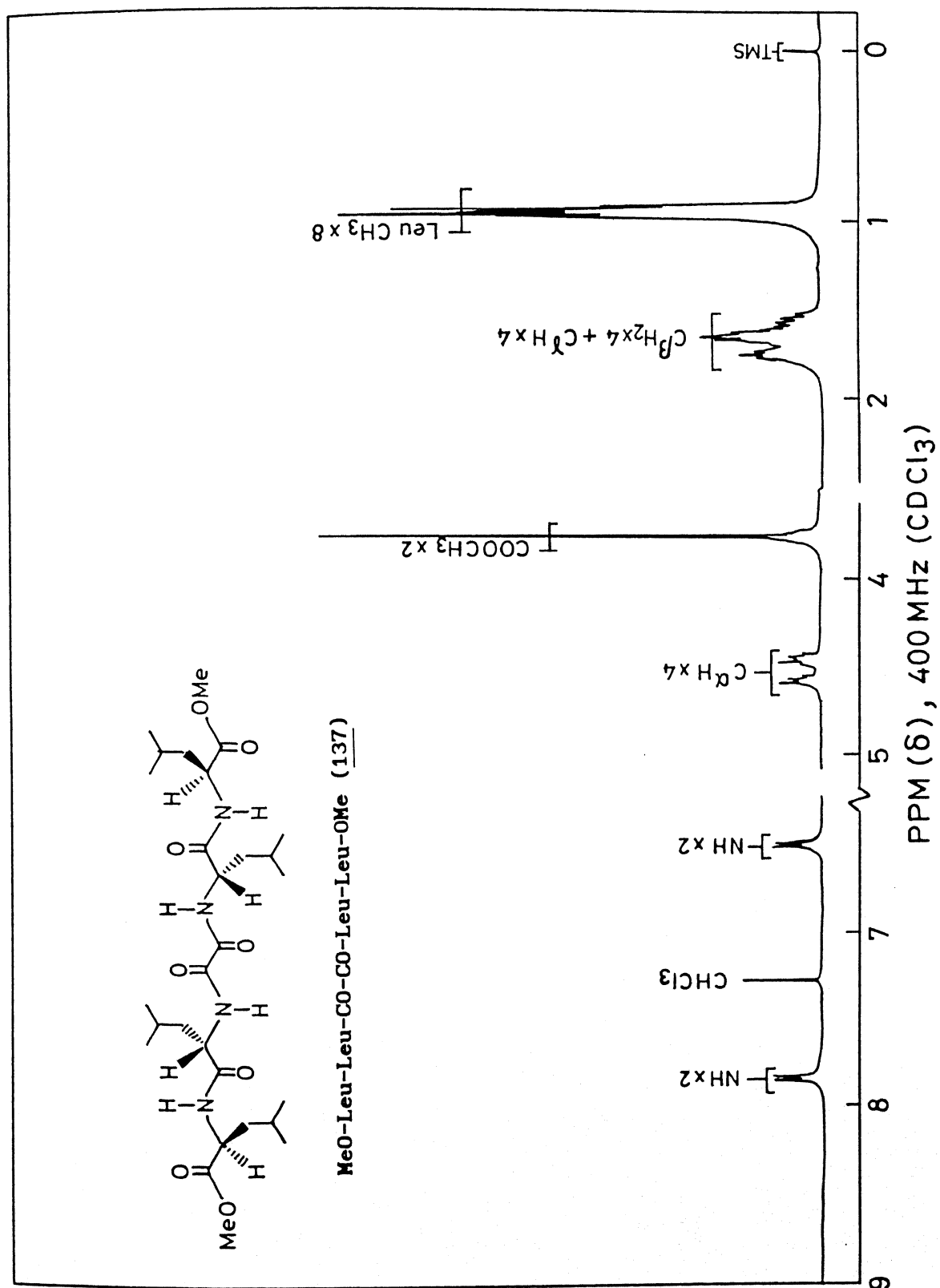


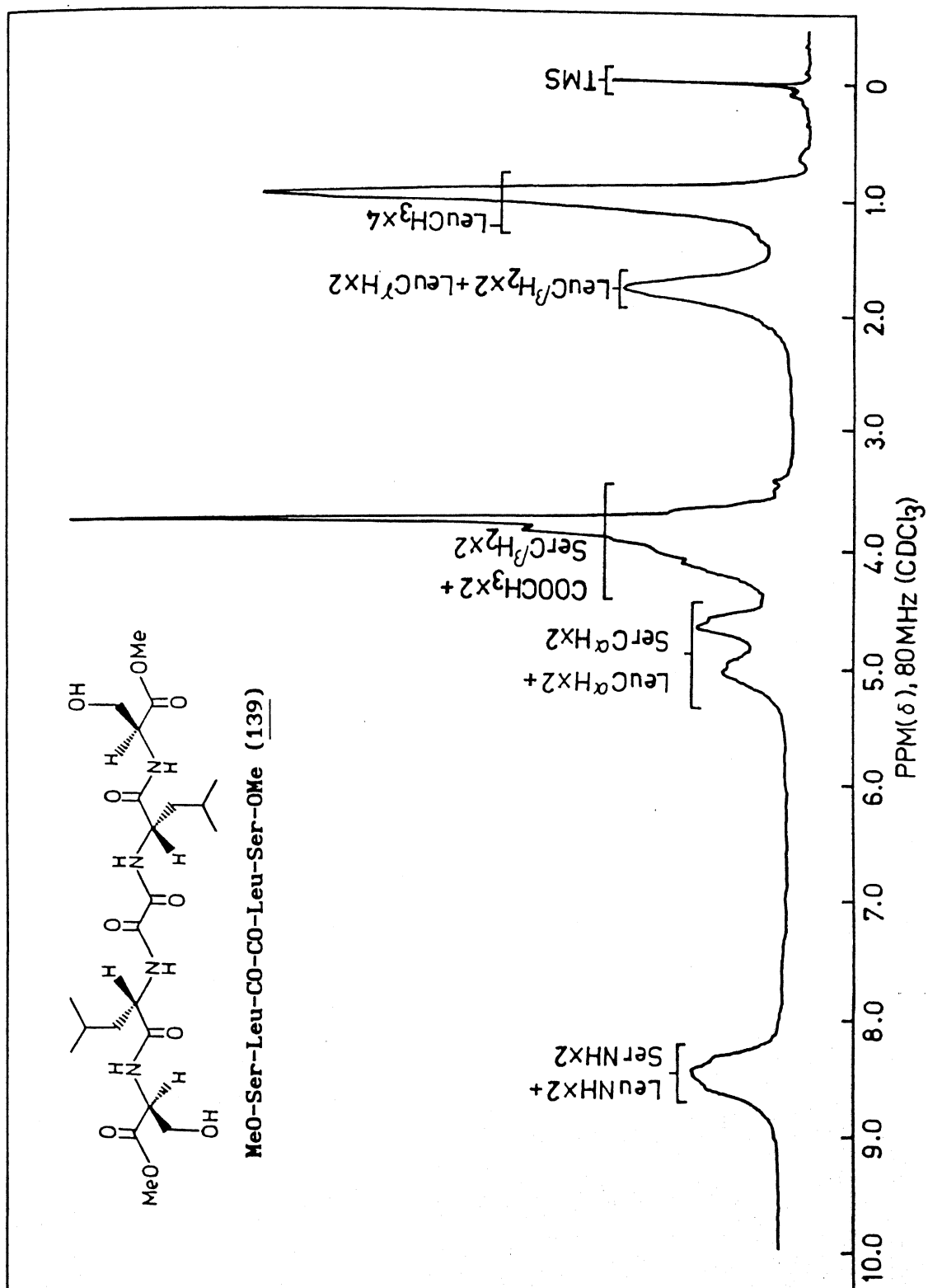


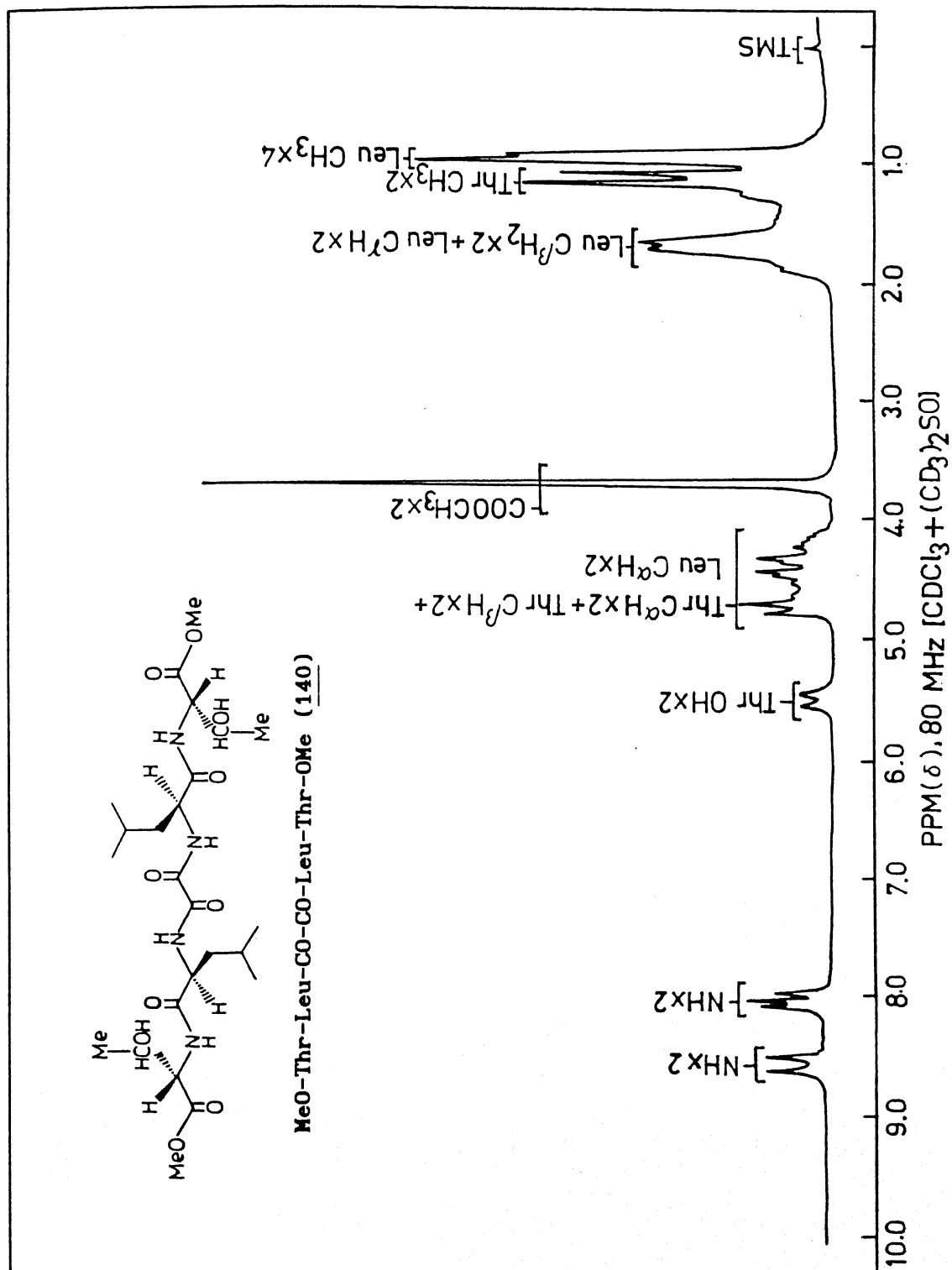


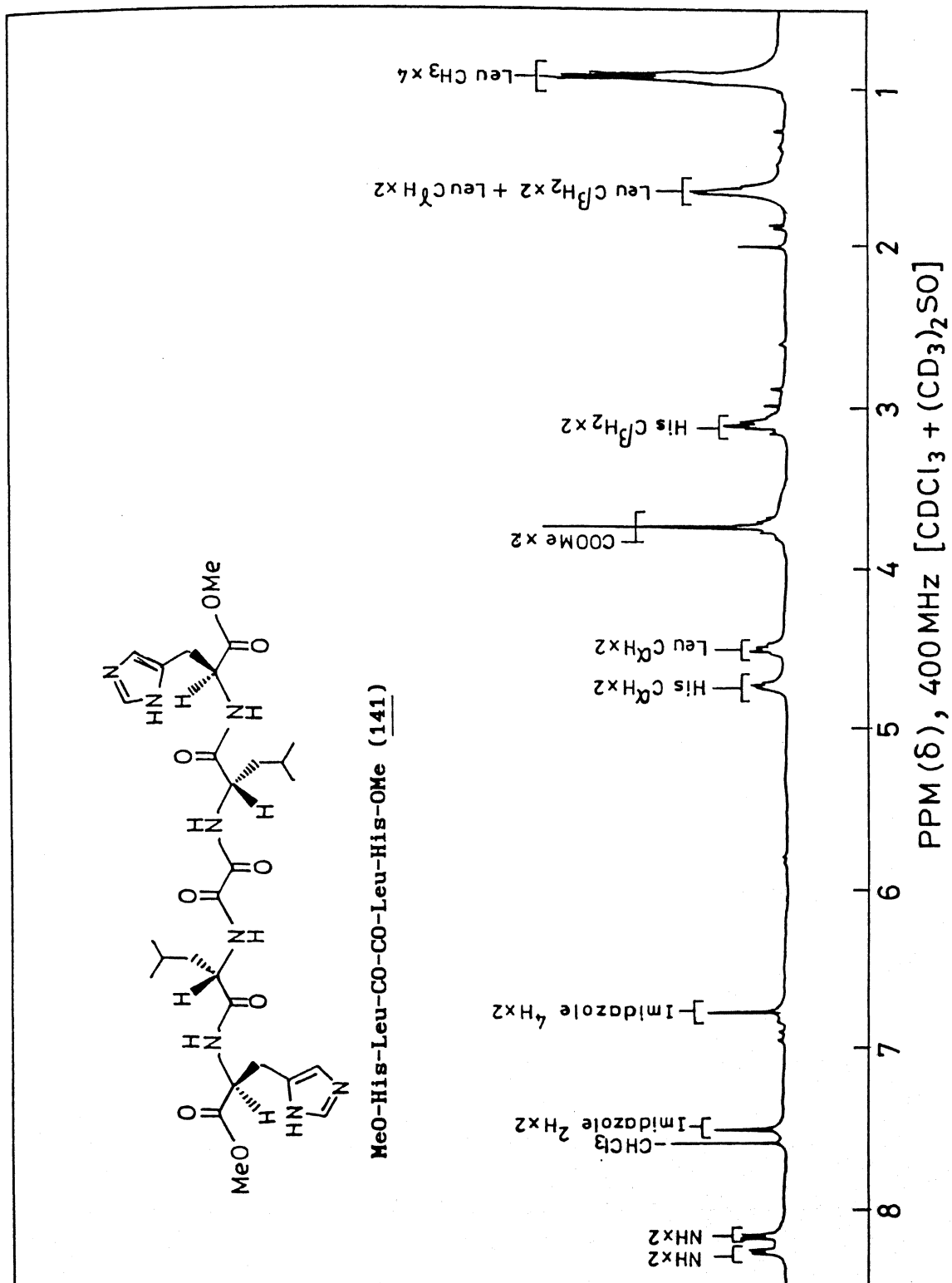


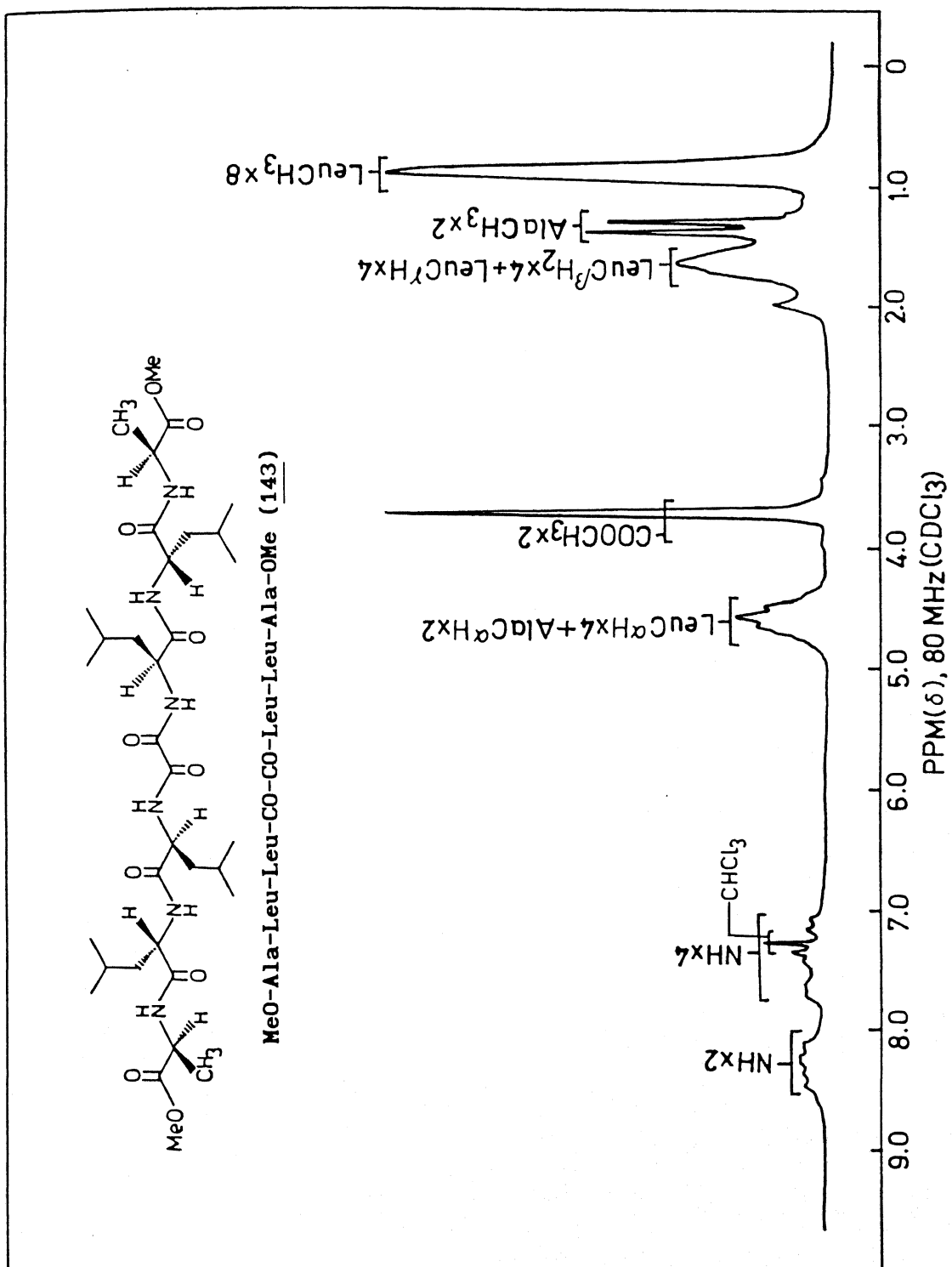


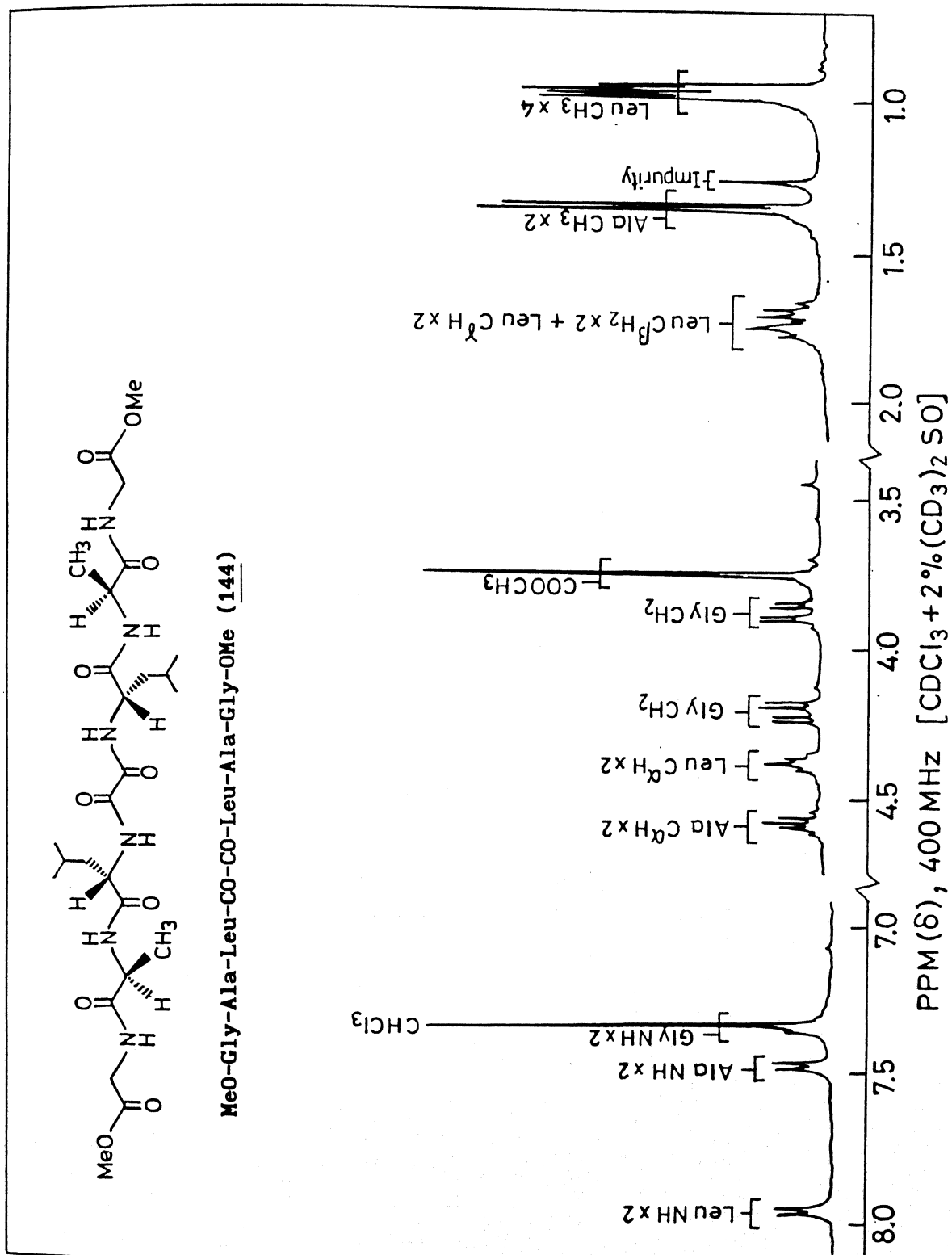


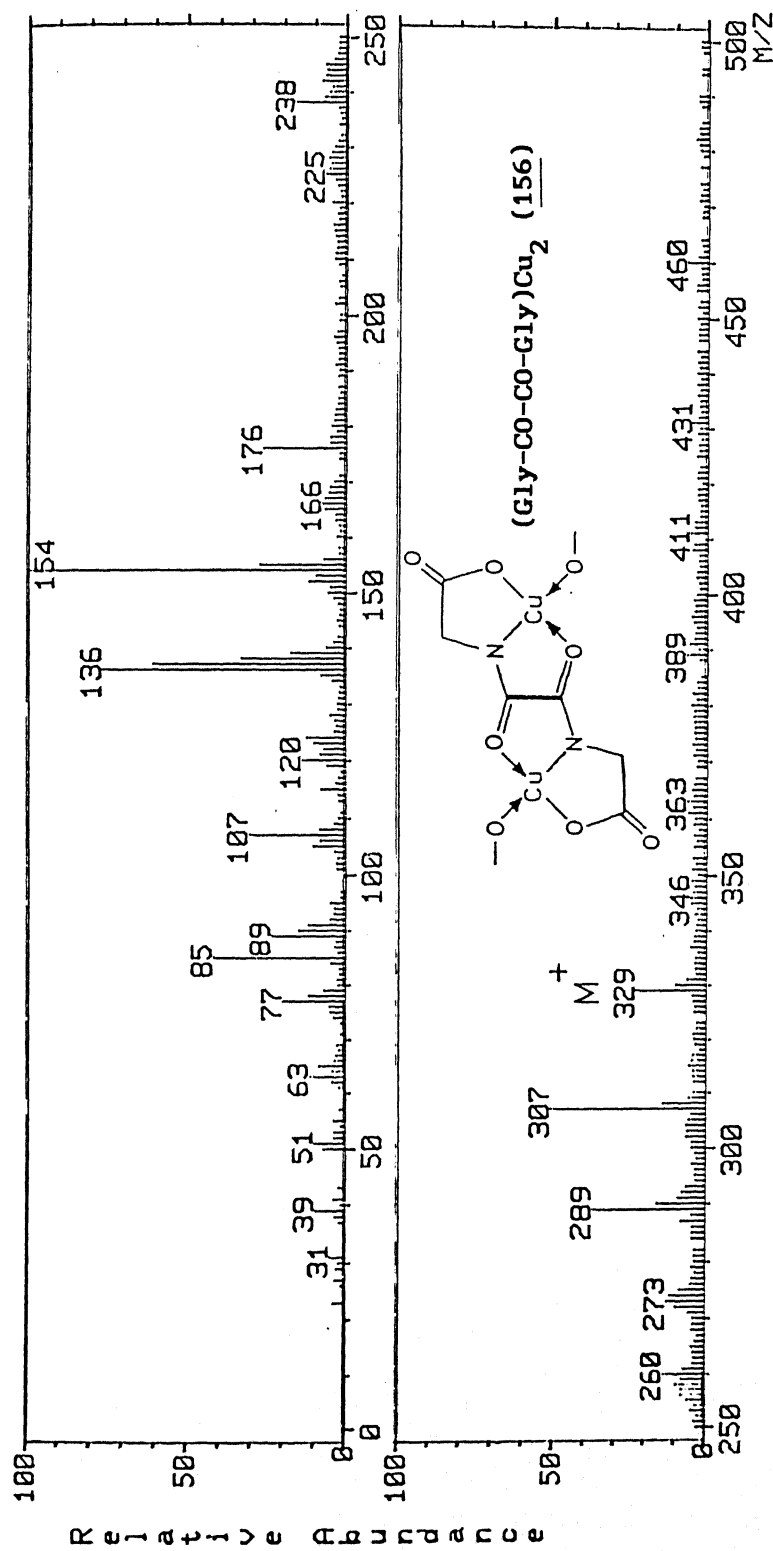


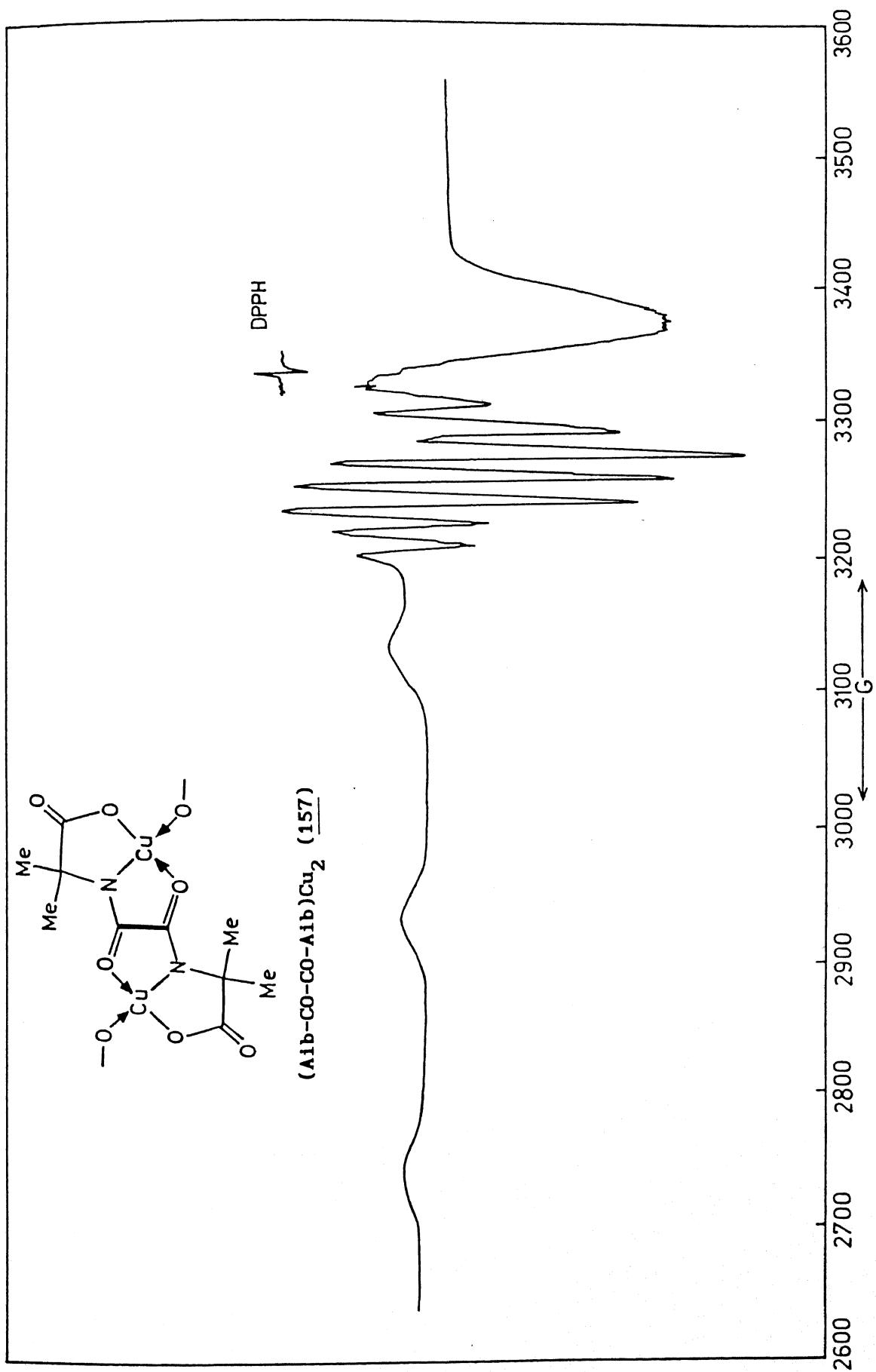


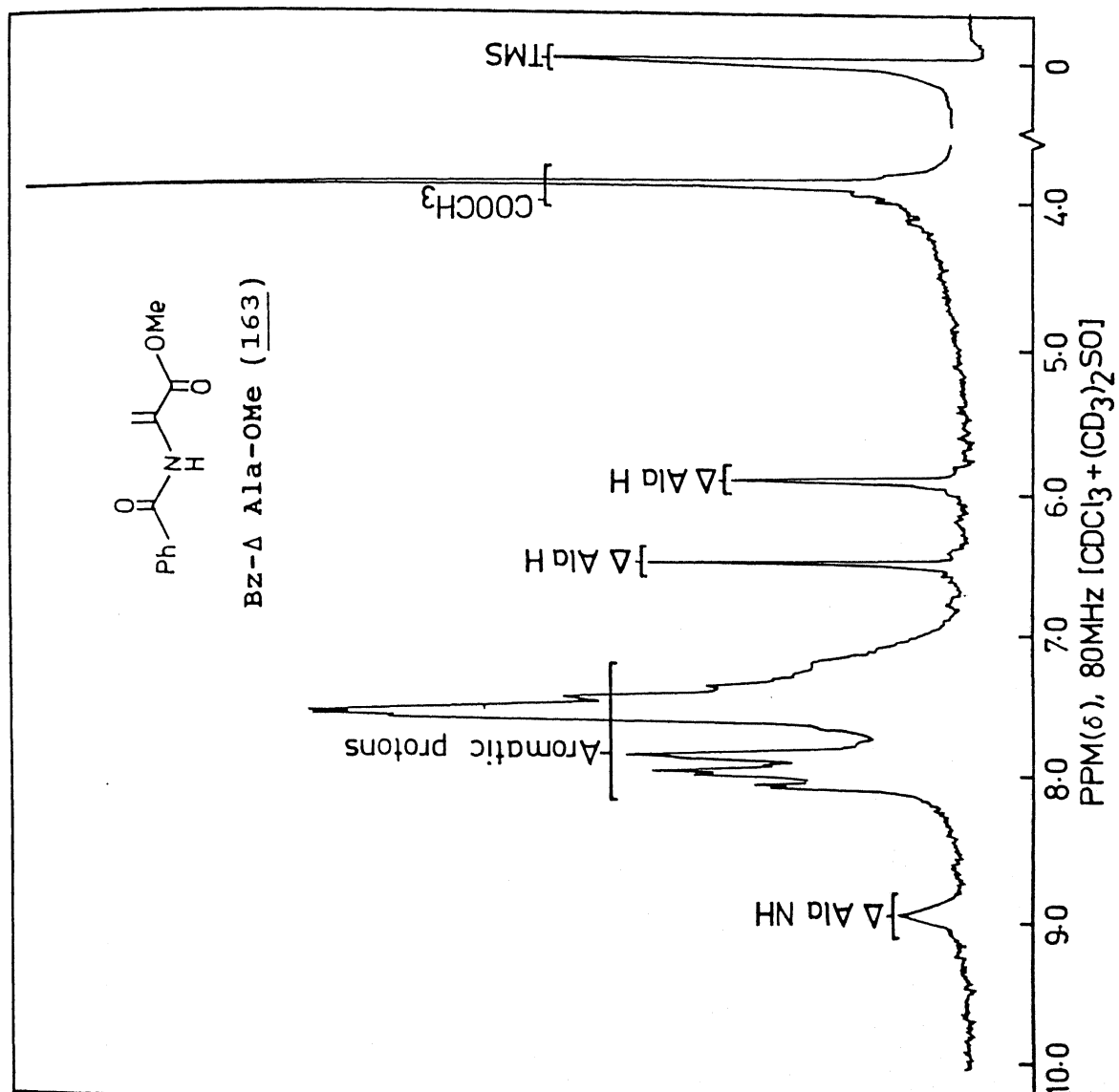


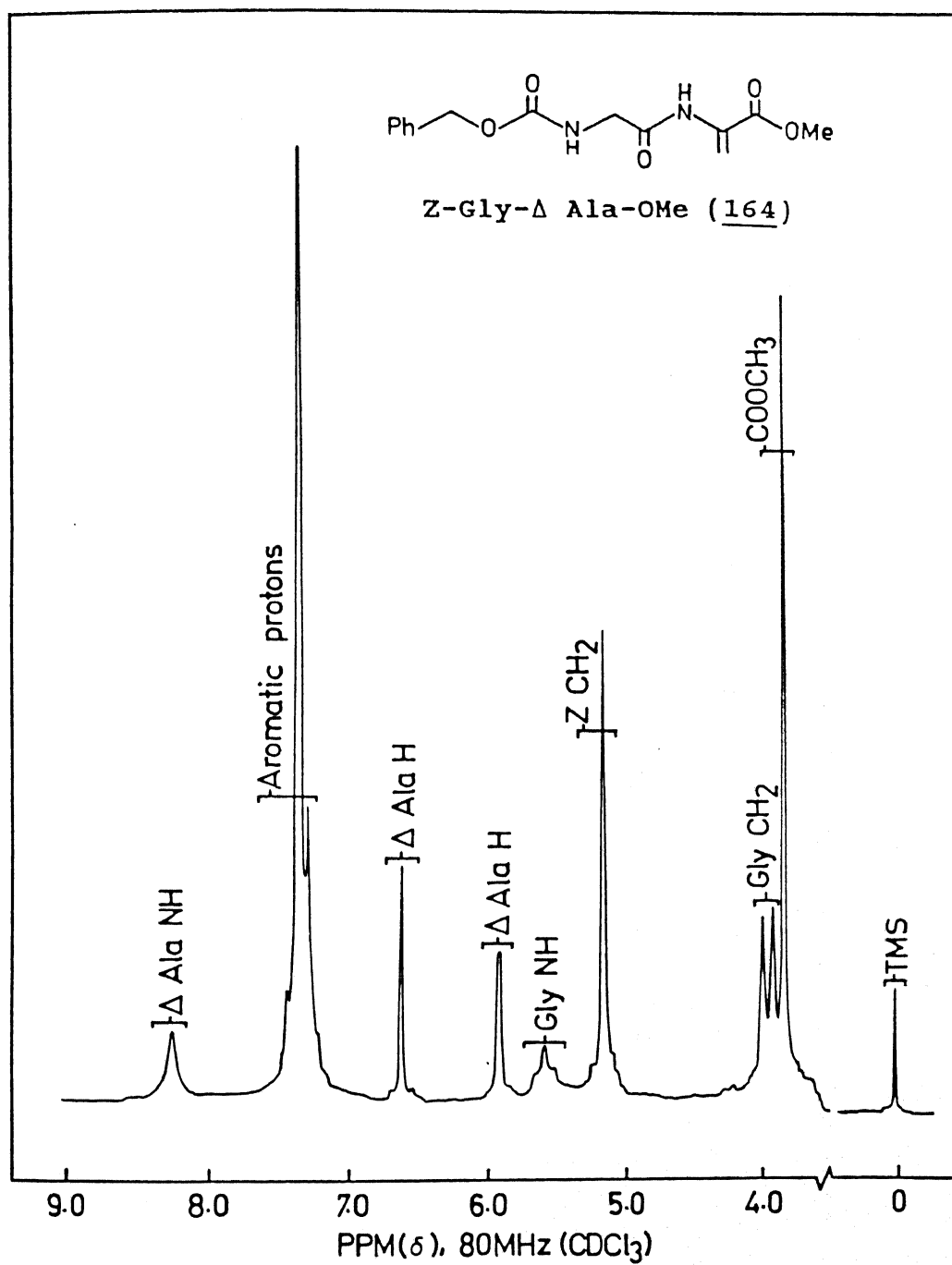


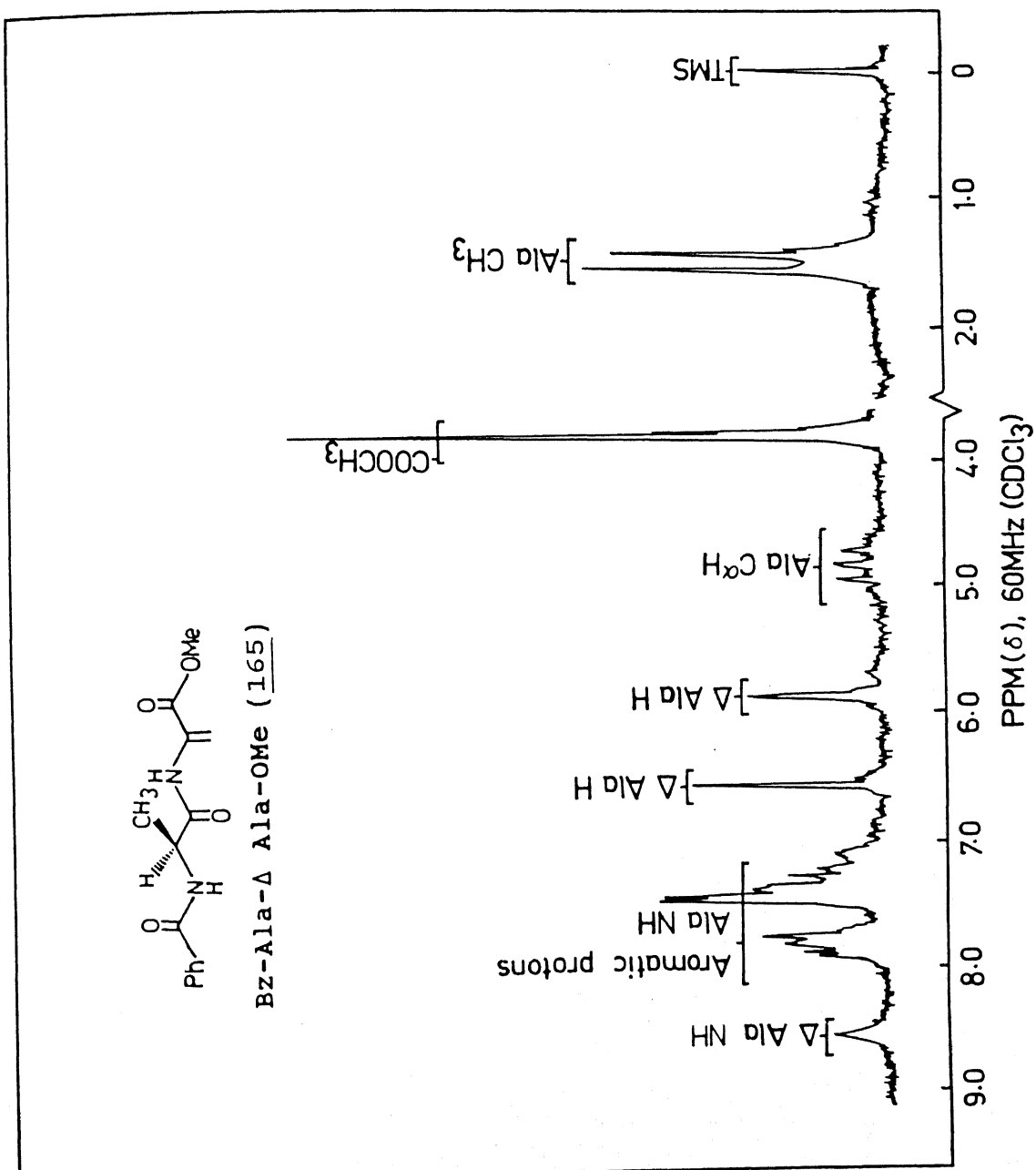


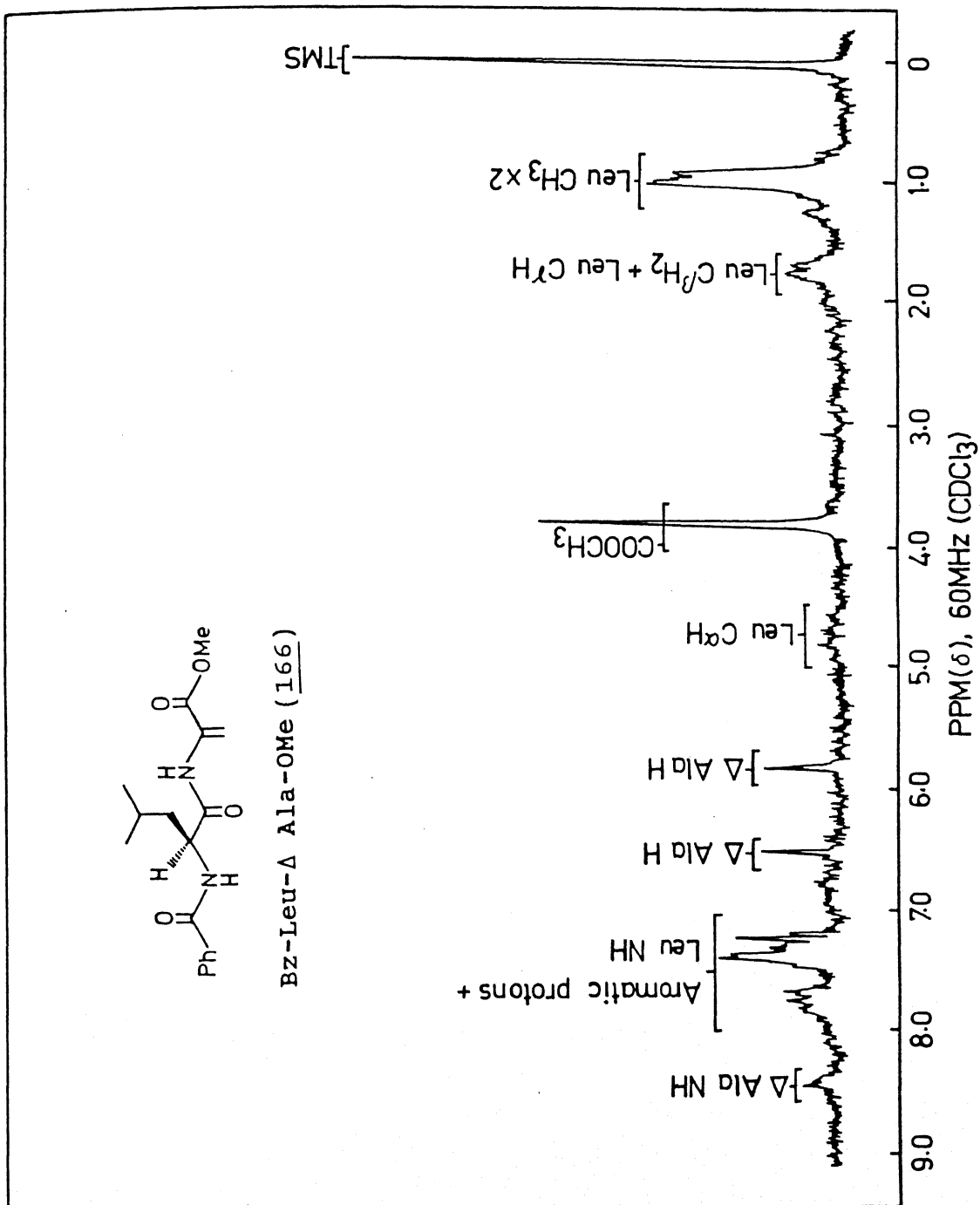


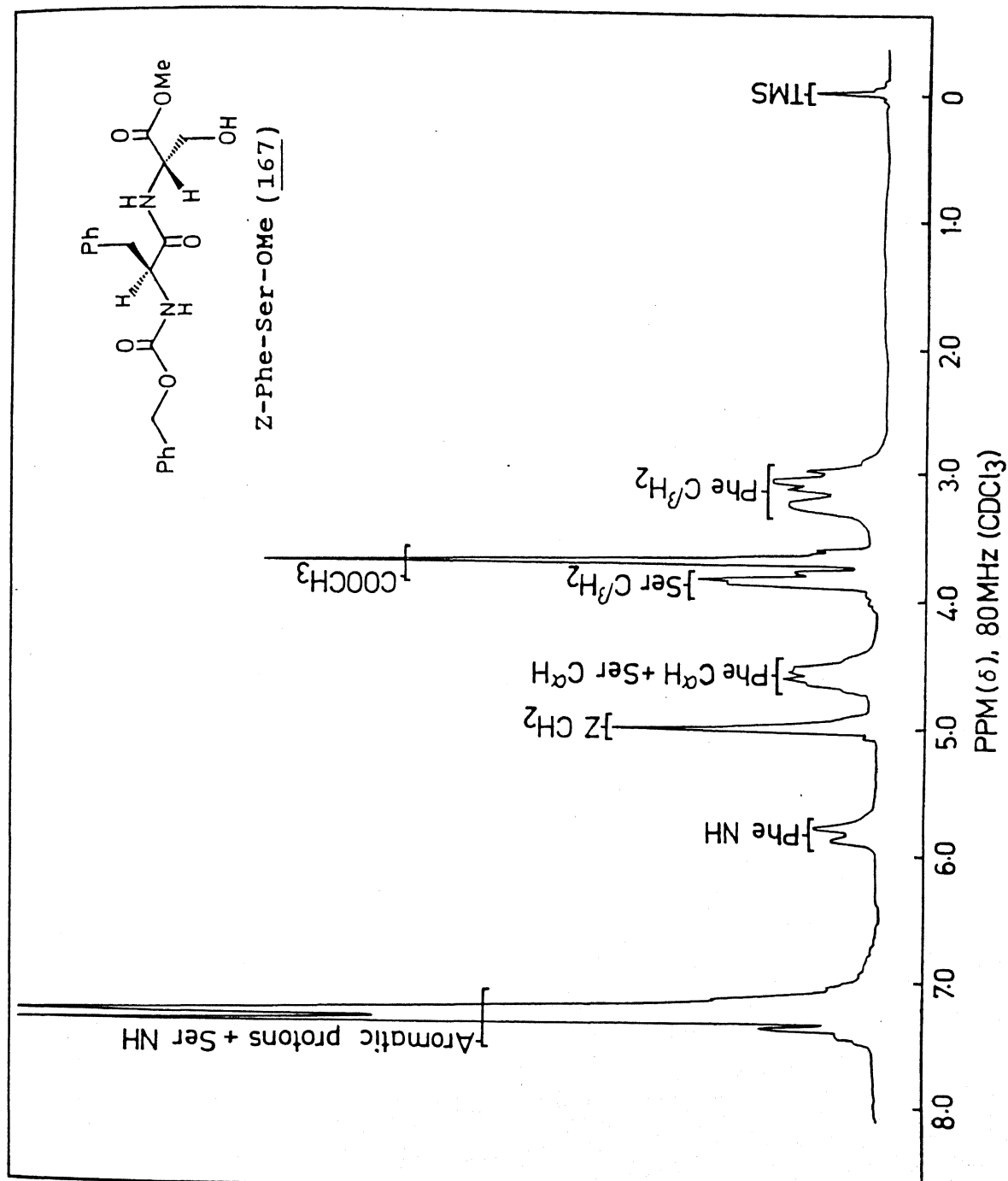


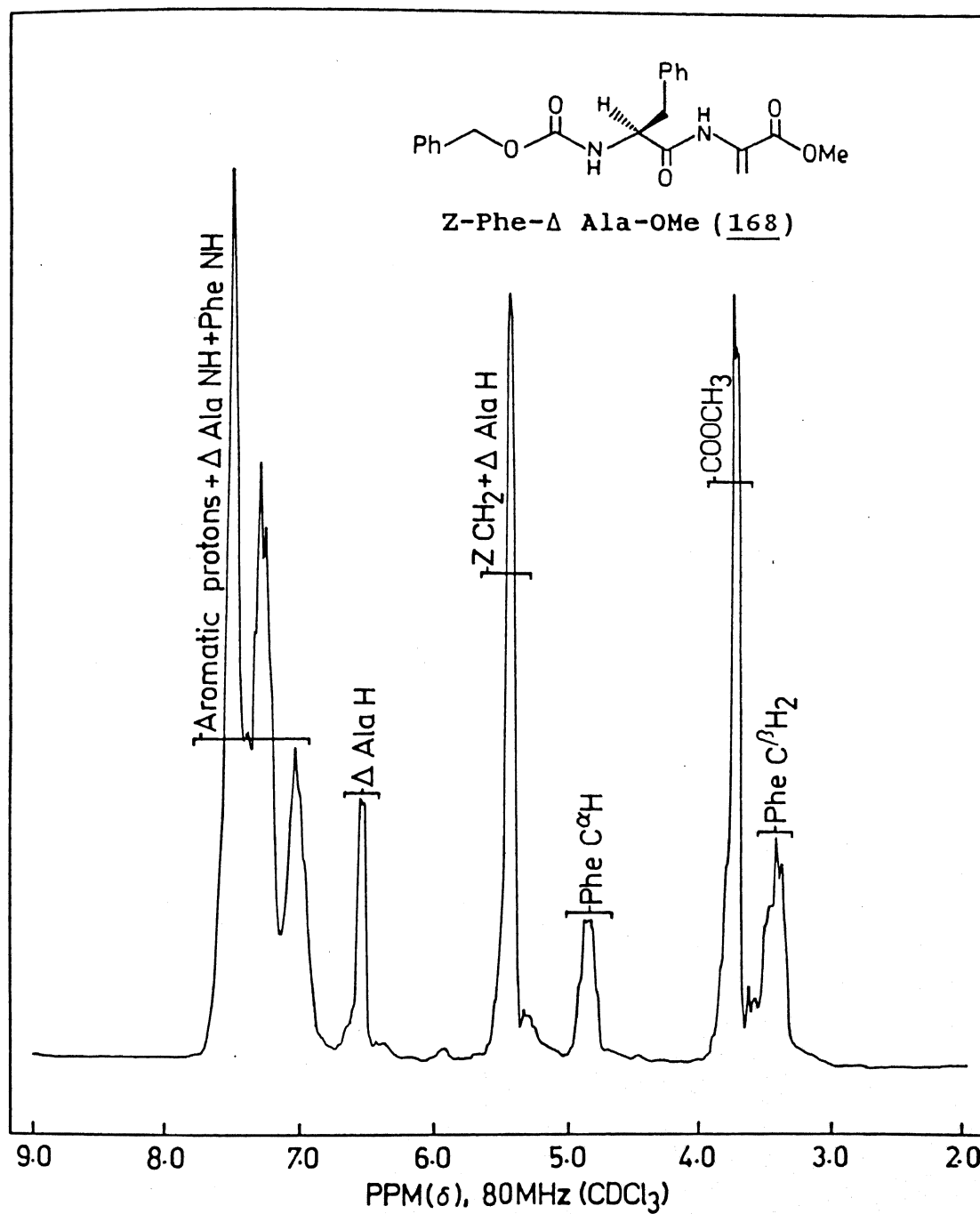


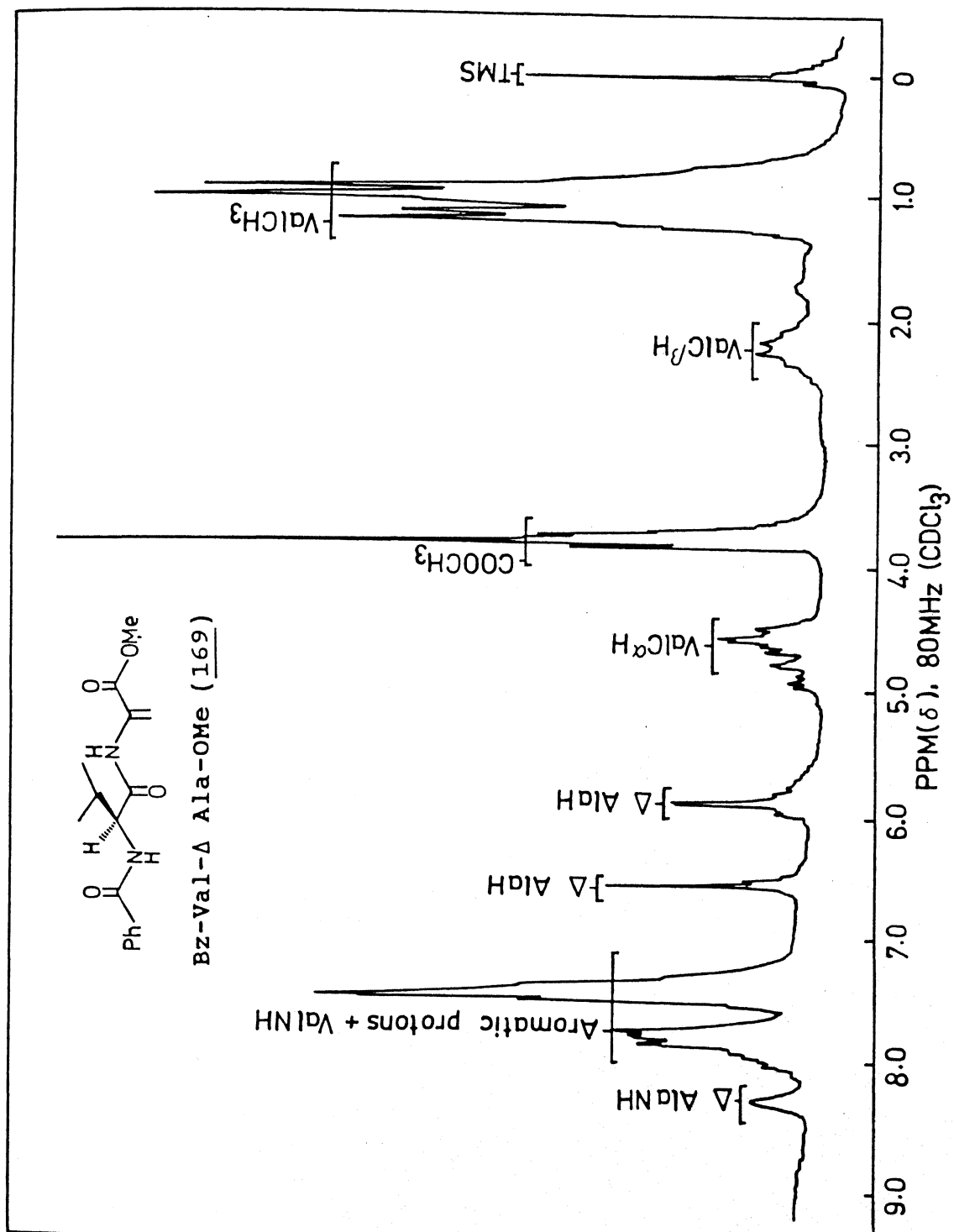


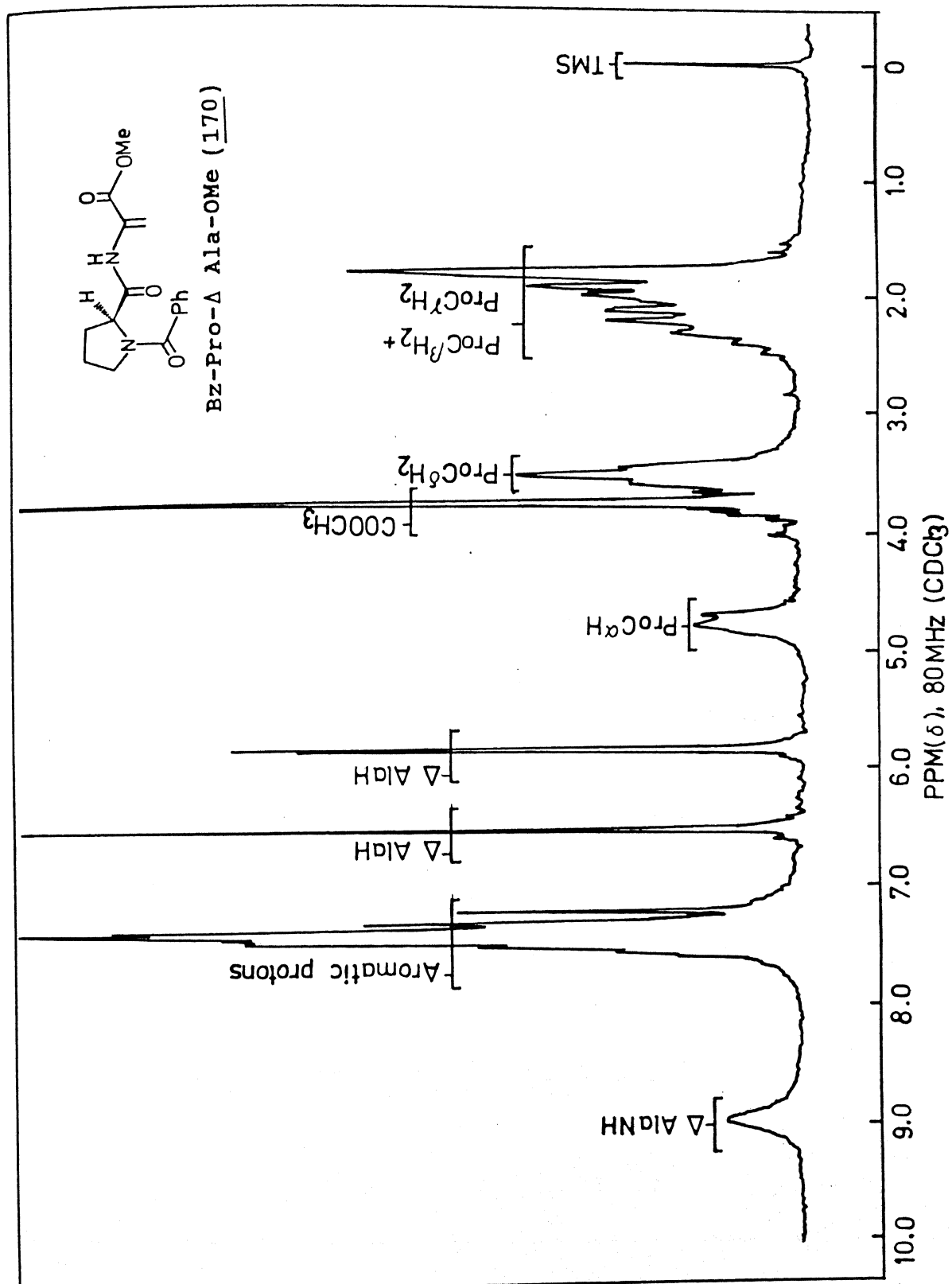


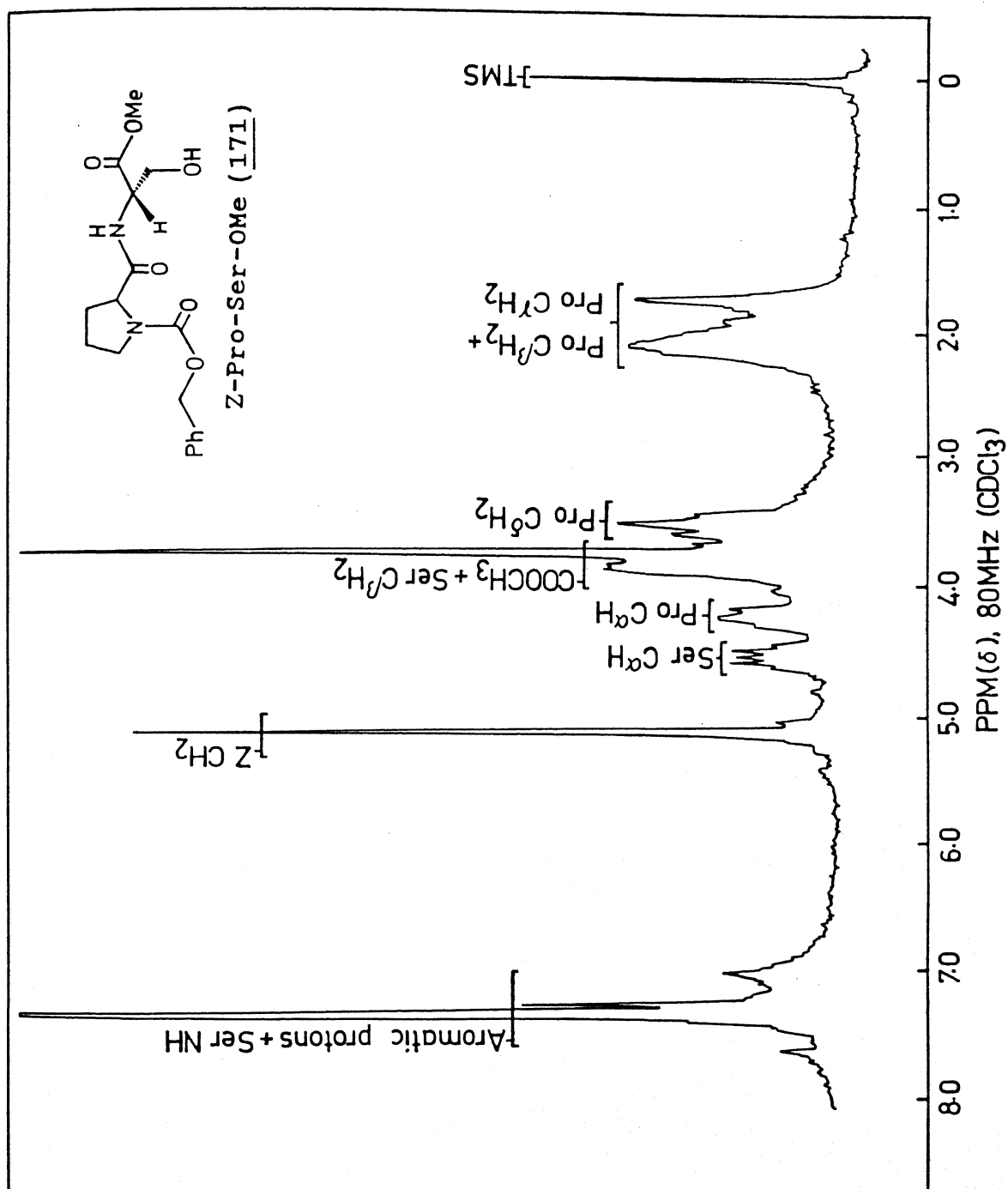


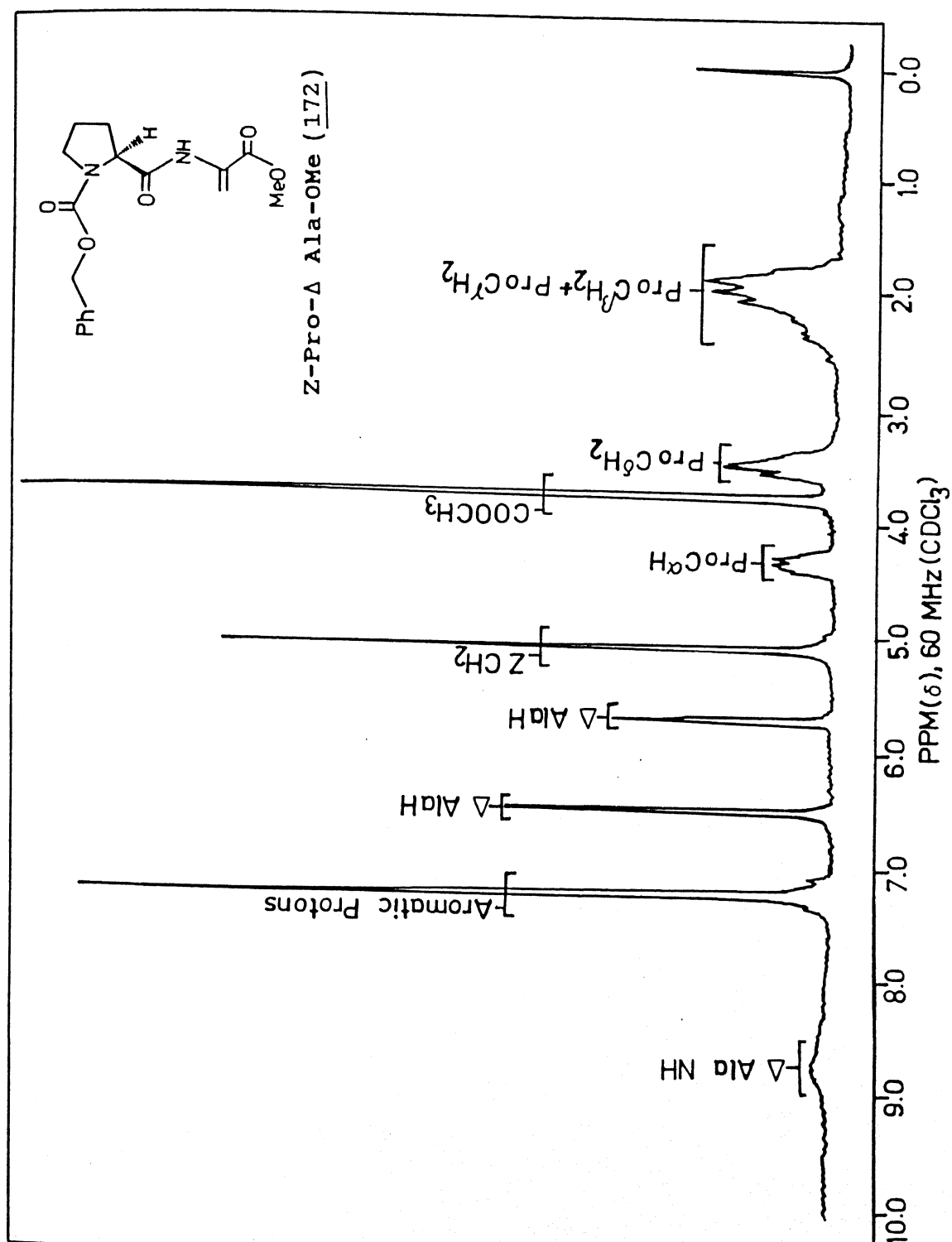


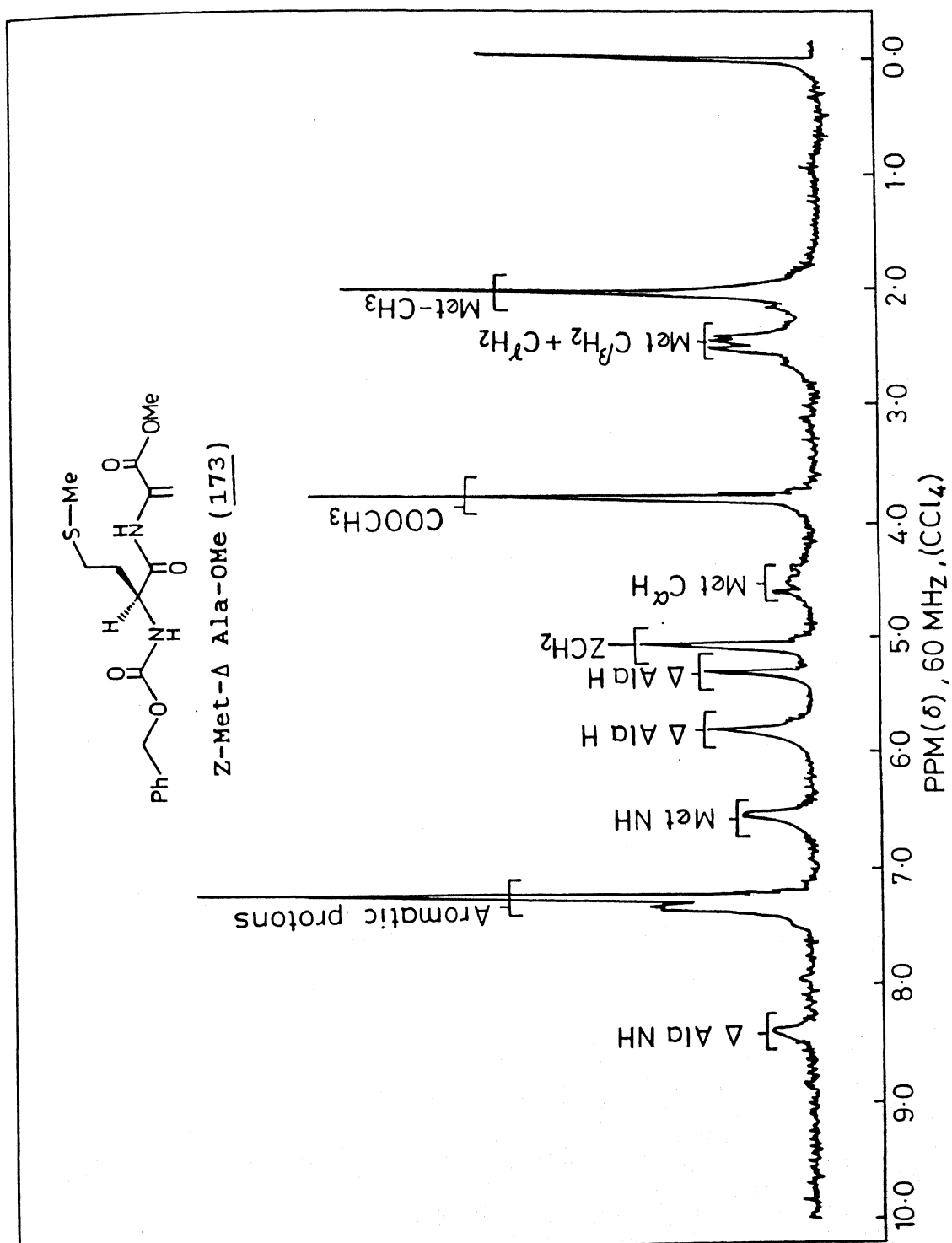


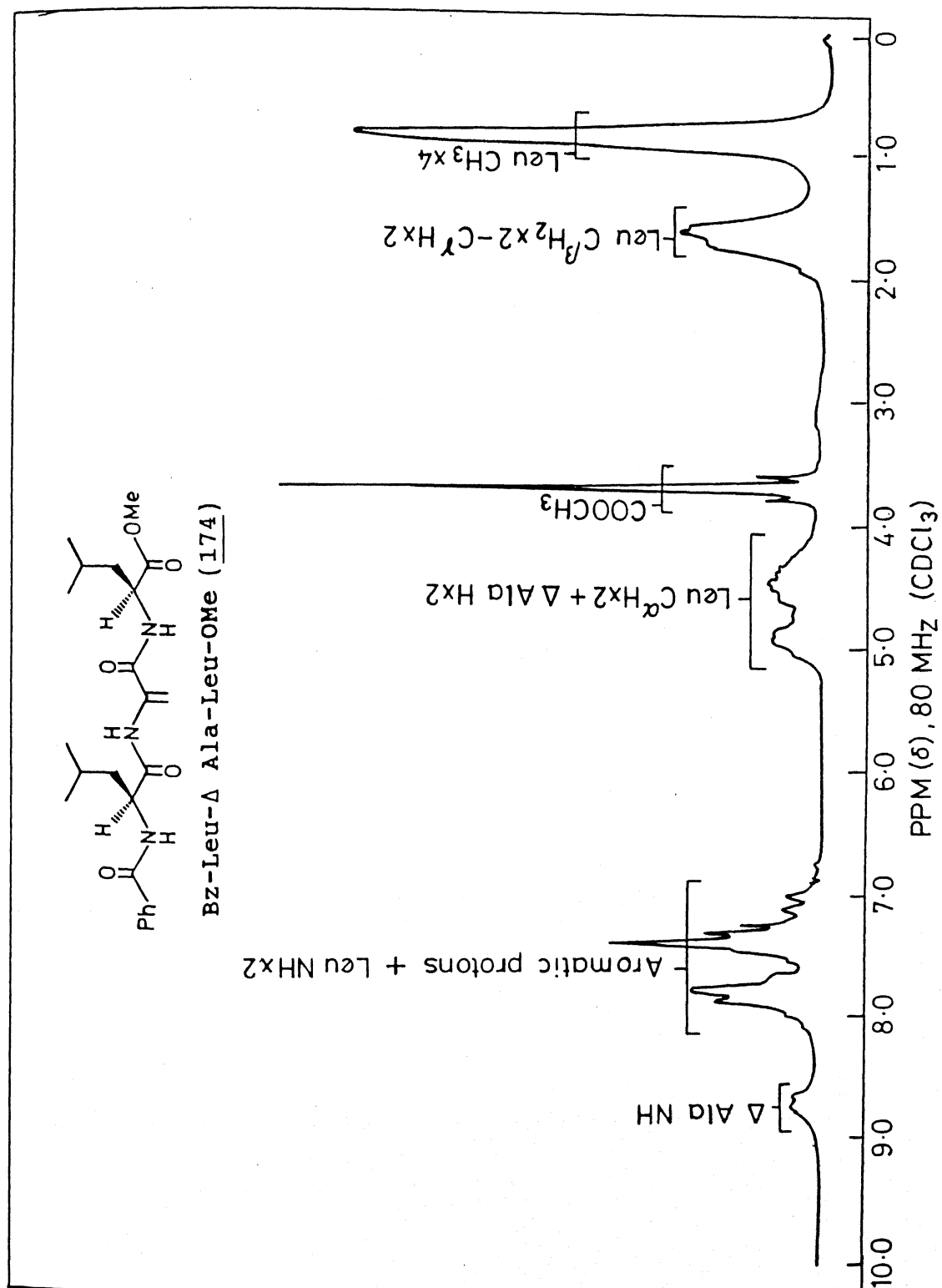


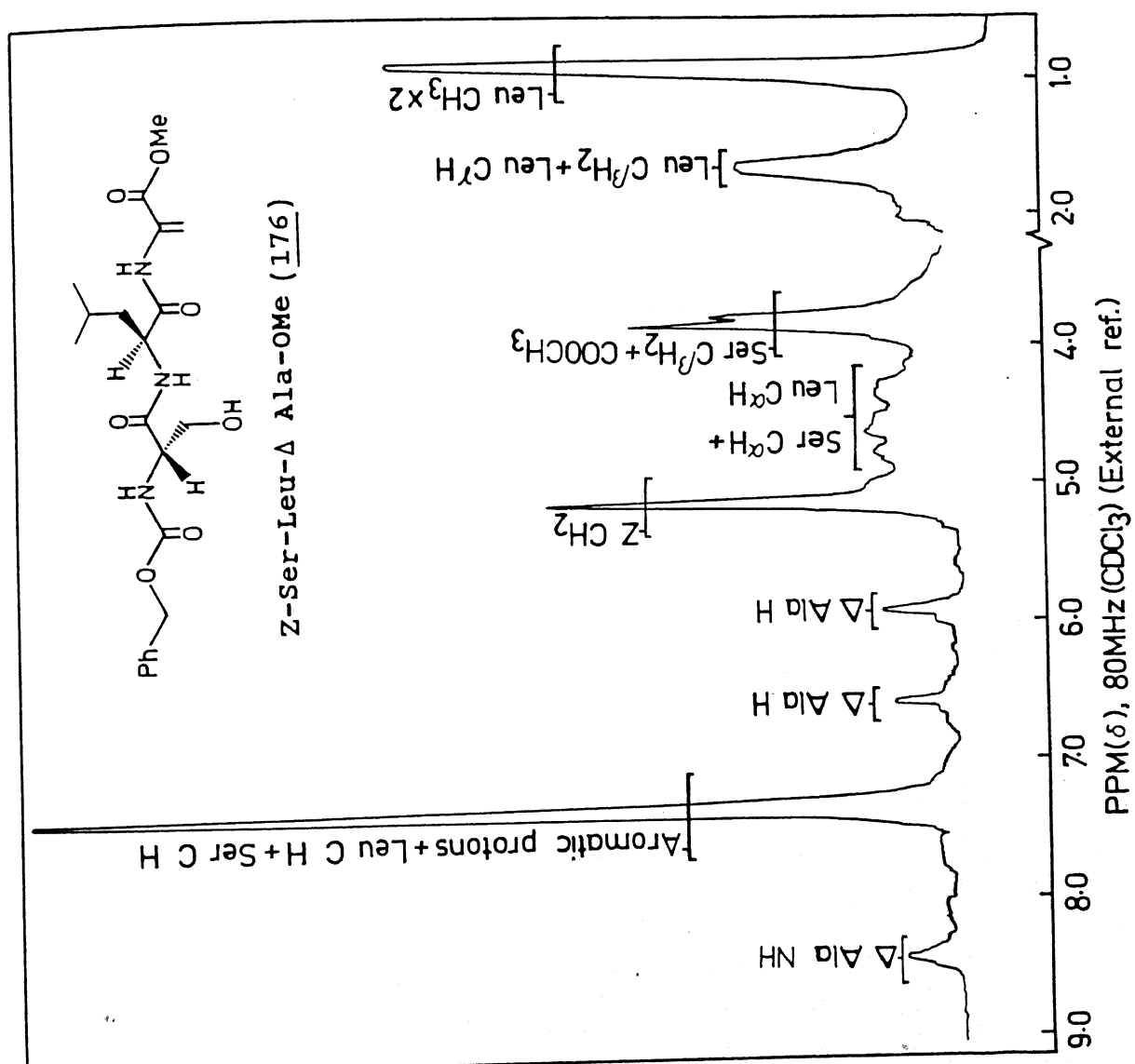


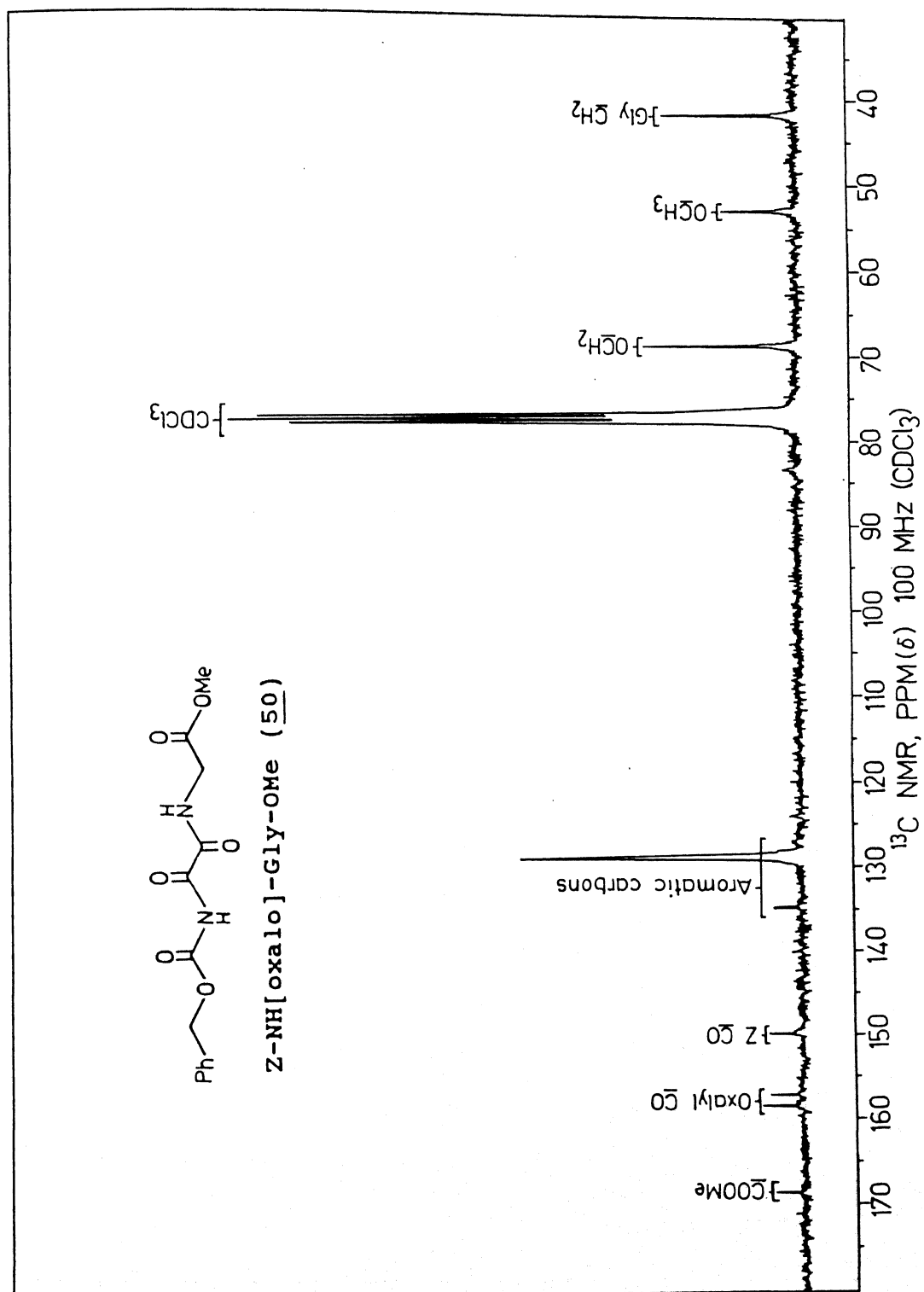


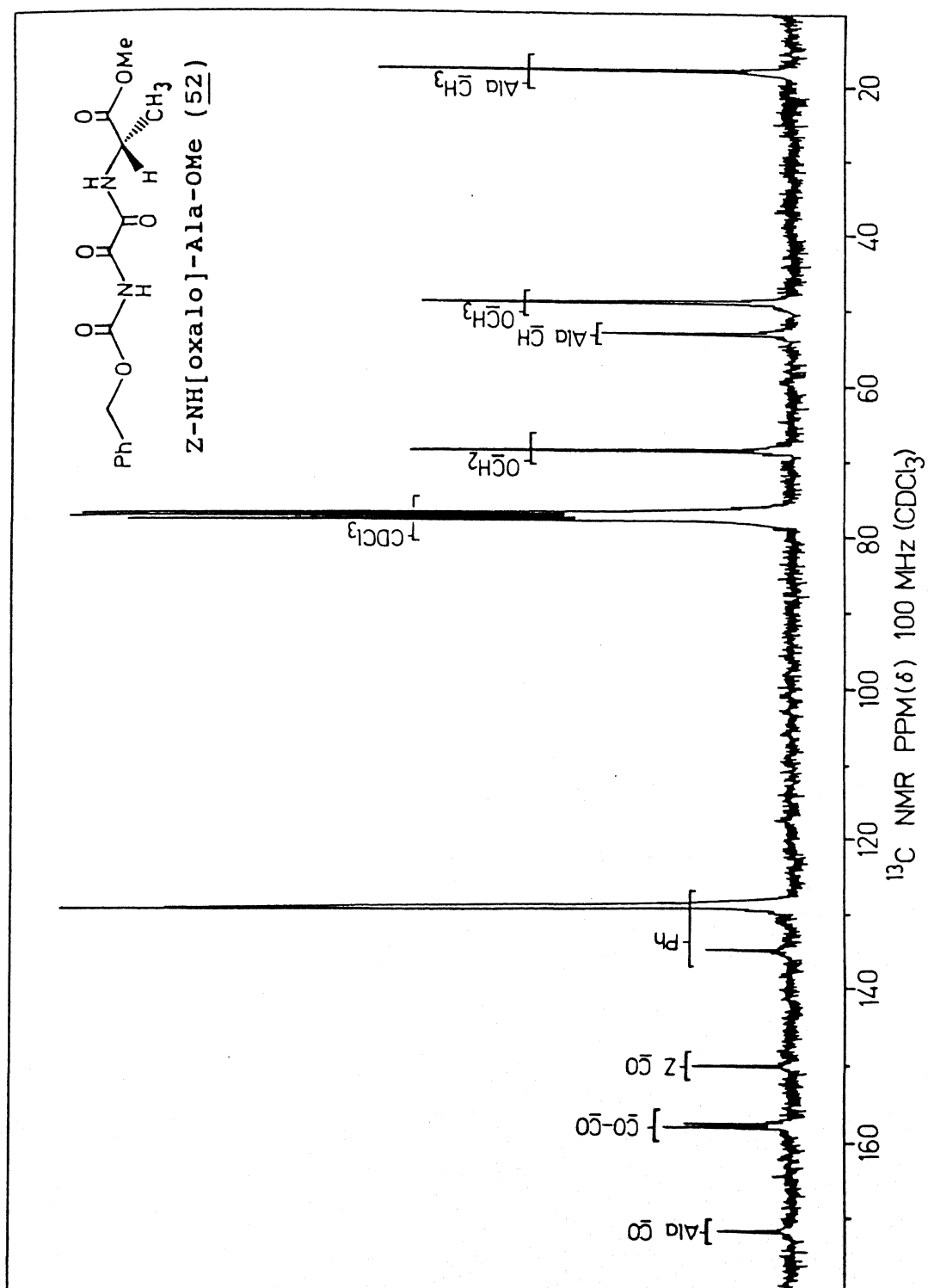












E. EXPERIMENTAL

General. All amino acids used were of L-configuration. ^1H NMR spectra were obtained on WM 400 Bruker instrument at 400 MHz, WP 80 Bruker instrument at 80 MHz and Hitachi R 600 at 60 MHz. The chemical shifts are recorded in ppm with TMS at 0.00 as internal standard or as external reference. IR spectra were recorded on PE 580/1600 FT instrument either as neat liquids or KBr pellets. Only prominent IR peaks are reported. Optical rotations were measured using an automatic JASCO digital polarimeter. FAB mass were recorded using a JEOL SX-120/DA-6000 instrument using argon (6KV, 10mA) as the FAB gas. The accelerating voltage was 10 KV and the spectra were recorded at room temperature with m-nitrobenzyl alcohol as the matrix. CD spectra were recorded on JASCO J-20 spectropolarimeter at $\sim 25^\circ\text{C}$ using 5mm path-length quartz cells, a time constant of 64sec and a chart speed of 4mm/minutes. Electronic spectra of solutions were recorded on a Perkin Elmer Lambda-2 UV-Vis spectrophotometer at 298°K using freshly purified solvents. EPR were recorded on a Varian E-109 spectrometer operating at the X-band using DPPH as the external standard. EPR spectra were taken at room temperature and at liquid nitrogen temperature (77°K). Elemental analysis were carried out in automatic C, H, N analysers. Silica gel G (Merck) was used for tlc. Column chromatography was done on silica gel (acme 100-200 mesh) columns, which were generally made from a slurry in benzene or ethyl acetate. Reactions were monitored wherever possible by tlc. The organic extracts were invariably dried over anhyd. MgSO_4 and solvents evaporated in vacuo.

General Procedures

N-Protection of α -Amino Acids:**(I) N-Benzylloxycarbonyl Amino Acids:**

To an ice-cooled and well stirred solution of the L-amino acid (100 mmol) in 25 mL of 4N NaOH (100 mmol) was added 30 mL of 4N NaOH (120 mmol) and 18.7 g (110 mmol) of benzyloxycarbonyl chloride alternately, in about 5 equal parts over a period of 20-30 min. (adjusting the mixture to pH~10 with excess alkali if necessary). The reaction mixture was left stirred for ~2h at 0°C, extracted with ether (2x30 mL) to remove excess carbobenzoxy chloride, the aqueous layer adjusted to pH~2 with 5N HCl, under cooling in an ice bath, extracted with EtOAc (3x30 mL), the organic layers dried (MgSO₄), solvents evaporated in vacuo and the residue obtained was crystallized from EtOAc/hexane to give N-Z protected amino acid in nearly quantitative yields.

(II) N-^tButyloxycarbonyl Amino Acid**(i) Boc-azide:**

To an ice-cooled mixture of Boc-carbazate (7.92g, 60 mmol), AcOH (6.91 mL) and water (9.66 mL), was added NaNO₂ (4.51g, 65 mmol) dissolved in minimum amount of water while maintaining the temperature below 5°C. Reaction mixture was left stirred at 0°C for ~30 min, diluted with H₂O (9.66 mL), extracted with ether (3x50 mL), the organic layers washed with cold water, aq. bicarbonate solution, dried (MgSO₄), solvents evaporated in vacuo (without heating) to give 7.0 g (81.6%) of Boc-azide as an oil. This was immediately used for the reaction.

(ii) N-^tButyloxycarbonyl Amino Acids (N-Boc-Amino Acids):

To an ice-cooled and well stirred solution of L-amino acid (40 mmol) in aq. NaOH (1.85g, 46.25 mmol in 24 mL H₂O) was added dioxane

(24 mL) followed by Boc-azide (60 mmol) and left stirred for 24h at room temperature. The reaction mixture was diluted with ice-water (~50 mL) and extracted with ether (2x25 mL). The aqueous phase was acidified with solid citric acid to pH~3, saturated with solid NaCl, extracted with EtOAc (3x50 mL), dried (MgSO_4), solvents evaporated in vacuo and the residue crystallized from EtOAc/hexane to give the N-Boc-amino acid in almost quantitative yields.

(III) N-Benzoyl Amino Acids:

To an ice-cooled and vigorously stirred solution of L-amino acid (30 mmol) in 2N NaOH (30 mL) was added, alternately, 5.45 mL (40 mmol) of benzoylchloride and 30 mL of 2N NaOH in ~10 equal portions, keeping the medium basic throughout the addition. The reaction mixture was left stirred for a further period of 0.5 h. at 0°C and at room temperature for ~2h, adjusted to pH~2 with 2N HCl, left aside at 0°C for 2h, the precipitated benzoyl derivative was filtered, washed with ice-cooled water, air dried overnight, digested with hot CCl_4 (2x15 mL) to remove benzoic acid and crystallized from methanol or ethylacetate to afford the N-benzoyl amino acids in nearly quantitative yields.

C-Protection Of α -Amino Acids: Preparation of Methyl Ester Hydrochlorides:

(IV) Dry HCl Method:

To a stirred suspension of L-amino acid (Ser, Gly, Ala, Leu, Pro, Asp; 100 mmol) in dry MeOH (~75 mL), dry HCl was passed first at room temperature until a clear solution was obtained, the passage of HCl continued at 0°C until saturation. The solvents were evaporated in vacuo and the residue obtained was crystallized from dry MeOH/ Et_2O , filtered, washed with dry Et_2O and dried in vacuo over KOH to give amino acid methyl ester hydrochloride in quantitative yields.

In the case of SerOMe.HCl, the residue, obtained after removal

of solvents, was redissolved in dry MeOH and again subjected to passage of dry HCl for ~1h, followed by evaporation of solvents and crystallization.

(V) SOCl₂ Method:

To a stirred and ice-cooled dry MeOH (35 mL), was added, in drops, SOCl₂ (4 mL, 58 mmol) followed by L-amino acid (Phe, Tyr, Aib, Met, Thr, Trp, 50 mmol). The reaction mixture was allowed to attain room temperature, refluxed for 2h, solvents evaporated and the residue on crystallization from dry MeOH/Et₂O gave products in more than 85% yields.

In the case of Thr and Met no refluxing was needed.

α -Amino Acid Coupling: Synthesis of Peptides:

All peptides were synthesised by solution phase method using either DCC/HOBt mediated coupling (Method A) or azide coupling (Methods B and C).

(VI) Method A:

Solid N-hydroxybenzotriazole (HOBt, 1 mmol) and dicyclohexylcarbodiimide (DCC, 1 mmol) were added sequentially at 0°C to a stirred solution of N-protected amino acid (1 mmol) in dry CH₂Cl₂ (20 mL) or in a mixture of dry DMF and CH₂Cl₂ in cases where solubility was poor in CH₂Cl₂. After a period of ~0.25h, the reaction mixture was admixed with the amino acid methyl ester, prepared at 0°C from the corresponding ester hydrochloride and triethylamine (1.2 mmol each) in dry CH₂Cl₂ or in a mixture of dry DMF and CH₂Cl₂. The combined mixture was left stirred at room temperature for 2 days, the precipitated DC urea was filtered, residue washed with CH₂Cl₂ (2x10 mL) and the combined filtrates washed sequentially with cold 2N H₂SO₄ (20 mL), water (20 mL) and saturated bicarbonate solution (20 mL). The organic extract was dried (MgSO₄) and evaporated in vacuo. The residue was, in

with H_2O (~10 mL), extracted with EtOAc (2x30 mL), organic extract washed with aq. bicarbonate solution and dried (MgSO_4). The residue obtained after solvent removal in vacuo was purified on a short column of silica gel using benzene/EtOAc as eluents.

Deprotection of N,C-Protected α -Amino Acids or Peptides:

C-Deprotection:

(IX) Hydrolysis of Methyl Ester:

A solution of N-protected L-amino acid methyl ester (1 mmol) in methanol (~4 mL) was cooled to 0°C , treated with cold aq. NaOH (2N, 4 mL) and stirred at room temperature until the starting material was consumed (tlc, ~4h). The reaction mixture was concentrated to half the volume (without heating) in vacuo, cooled in ice and acidified (pH~3) with 2N H_2SO_4 , saturated with solid NaCl and extracted with EtOAc (3x30 mL), dried (MgSO_4) and evaporated in vacuo. The residue was directly used for the next reaction.

In the case of hydrophobic amino acids, e.g. Trp, Tyr, Leu and Aib the precipitated N-protected amino acid or peptide was filtered, washed with water and directly crystallized from hot methanol or EtOAc.

N-Deprotection:

(X) Removal of Boc-group:

N-Boc protected amino acid or peptide methyl ester (1 mmol) was dissolved in dry CH_2Cl_2 (~3 mL), cooled to 0°C , admixed with CF_3COOH (1 mL), stirred at 0°C until the starting material was consumed (tlc, ~2h). The solvents were removed under reduced pressure without heating and the residue was thoroughly dried under high vacuum. The residual trifluoroacetate salt was directly used for the next reaction.

In the case of hydrophobic amino acids or peptides (e.g.

Leu-Leu), the trifluoroacetate salt was neutralized with cold aq. sodium carbonate (5%) and extracted with EtOAc (2x30 mL) and the dried (MgSO_4) extract was directly used for coupling reaction.

(XI) Removal of Z-group:

N-Z protected amino acid or peptide methyl ester (1 mmol) was dissolved in ethyl acetate (~10 mL), admixed with palladized charcoal (5%, w/w, 0.25g) and subjected to hydrogenolysis (under slightly positive pressure of hydrogen). The progress of the reaction was followed by tlc (~4-6h). The reaction mixture was filtered (sintered funnel with silica gel as the bottom layer) and the filtrate was directly used for coupling.

(XII) Hydrazide formation of α -Amino Acids or Peptides from Methyl Ester:

N-Protected amino acid or peptide methyl ester (1 mmol) was dissolved in minimum amount of dry ethanol, admixed with hydrazine hydrate (1.5-2 mmol) and stirred at room temperature for 24h. The precipitated solid was filtered, washed with chilled ethanol, dried in vacuo and directly used for the next reaction.

(XIII) Amide Formation from Amino Acid or Peptide Methyl Esters:

N-Protected amino acid or peptide methyl ester was dissolved in dry methanol, cooled to 0°C and a slow stream of dry NH_3 was passed through the stirring solution of the starting ester (tlc, ~2-12h). The reaction mixture was kept in fridge overnight, solvents evaporated under reduced pressure and the residue was directly crystallized from hot methanol or EtOAc.

All the procedures were used as described above unless specified otherwise and the methods used for synthesising protected amino acids or peptides is given in the brackets. Percentage yields of the products are given in brackets.

(1) N-Benzoyl L-Serine Methyl Ester (Bz-Ser-OMe, 1):

(i) Serine methyl ester hydrochloride (H-Ser-OMe.HCl): (Method IV)(95%)

mp.: 166°C (lit.¹⁰⁶ mp. 166°C).ir : ν_{max} (KBr) cm^{-1} : 3380, 1730 (ester).(ii) Bz-Ser-OMe(1):

To an ice-cooled and vigorously shaken solution of Ser-OMe.HCl (6g, 38.4 mmol) in satd. aq. NaHCO_3 (300 mL) was added, in drops, Bz-Cl (4.5 mL, 38 mmol). The reaction mixture was left stirred for 3h, maintaining the pH ~ 9 , by addition of aqueous saturated NaHCO_3 , extracted with Et_2O (3x100 mL), dried, evaporated and the residue on crystallization from dry PhH/hexane, gave, 7.12g (83%) of Bz-Ser-OMe, mp. 86° (lit.¹⁰⁷ mp. 86°C).

ir : ν_{max} (KBr) cm^{-1} : 3430 (OH), 3300 (NH), 1740 (ester), 1620 (amide I), 1530 (amide II).

nmr : $\delta(\text{CDCl}_3)$: 3.15 (1H, br, Ser OH), 3.71 (3H, s, COOCH_3), 3.95 (2H, dd, Ser $\text{C}^{\beta}\text{H}_2$), 4.80 (1H, m, Ser $\text{C}^{\alpha}\text{H}$), 7.11-8.05 (6H, m, NH, aromatic protons).

(2) o-NO₂Benzoyl L-Serine Methyl Ester (o-NO₂Bz-Ser-OMe, 3):

To an ice-cooled, stirred suspension of Ser-OMe.HCl (2.32g, 15 mmol) in dry CH_2Cl_2 (20 mL) was added triethylamine (5.5 mL, 40 mmol) followed by o-NO₂benzoyl chloride (1.85g, 10 mmol). The reaction mixture was left stirred for $\sim 1\text{h}$ at 0°C , washed with ice-cooled 2N H_2SO_4 (10 mL), water (10 mL), satd. aq. bicarbonate (10 mL), dried (MgSO_4), evaporated in vacuo, the residue crystallized from EtOAc/hexane to give 1.34 g (50%) of o-NO₂Bz-Ser-OMe, as pale yellow needles, mp. 92°C .

ir : ν_{max} (KBr) cm^{-1} : 3568 (OH), 3283 (NH), 1743 (ester), 1634 (amide I), 1611, 1573 (amide II), 1522 (NO_2), 1356 (NO_2).

nmr : $\delta(\text{CDCl}_3)$: 3.84 (3H, s, COOCH_3), 4.09 (2H, m, Ser $\text{C}^{\beta}\text{H}_2$), 4.81 (1H, m, Ser $\text{C}^{\alpha}\text{H}$), 6.79 (1H, br, NH), 7.34-8.28 (4H, m,

aromatic protons).

(3) N-Benzyloxycarbonyl Glycyl-L-Serine Methyl Ester (Z-Gly-Ser-OMe, 5):



(i) N-Benzyloxycarbonyl glycine (Z-Gly-OH): (96%)

mp. : 117-118°C (lit.¹⁰⁸ mp. 119-120°C)

(ii) Z-Gly-Ser-OMe (5): (94%)

mp. : 94-95°C (lit.¹⁰⁹ mp. 96°C)

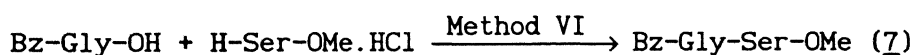
ir : ν_{max} (KBr) cm^{-1} : 3395 (OH), 3310 (NH), 1733 (ester), 1718, 1688 (amide I), 1657 (amide I), 1540 (amide II), 1513 (amide II).

nmr : $\delta(\text{CDCl}_3)$: 3.73 (5H, s + m, COOCH_3 + Ser C^βH_2), 3.89 (2H, d, $J=5$ Hz, Gly CH_2), 4.60 (1H, m, Ser C^αH), 5.09 (2H, s, Z CH_2), 6.00 (1H, t, exchangeable with D_2O , Gly NH), 7.32 (6H, s, Ser NH + aromatic protons).

anal: Found: C, 54.33; H, 5.65; N, 8.87 %

Calc. for $\text{C}_{14}\text{H}_{18}\text{N}_2\text{O}_6$: C, 54.19; H, 5.81; N, 9.03 %

(4) N-Benzoyl Glycyl-L-Serine Methyl Ester (Bz-Gly-Ser-OMe, 7):



(i) N-Benzoyl glycine (Bz-Gly-OH): (92%)

mp. : 185-186°C (lit.¹¹⁰ mp. 189.5-191.5°C).

(ii) Bz-Gly-Ser-OMe (7): (70%)

mp. : 82-84°C

ir : ν_{max} (KBr) cm^{-1} : 3360 (OH), 3285 (NH), 1730 (ester), 1655 (amide I), 1630 (amide I), 1555 (amide II).

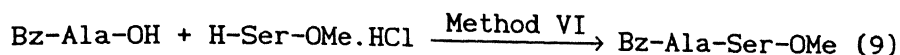
nmr : $\delta[\text{CDCl}_3 + (\text{CD}_3)_2\text{SO}]$: 3.80 (3H, s, COOCH_3), 3.91 (2H, m, Ser C^βH_2), 4.14 (2H, d, $J=5$ Hz, Gly CH_2), 4.60 (1H, m, Ser C^αH), 7.20-8.25 (7H, m, Ser NH + Gly NH + aromatic protons).

ms : m/z : 281 (MH)⁺.

anal: found: C, 55.44; H, 5.98; N, 10.09 %

Calc. for $C_{13}H_{16}N_2O_5$: C, 55.71; H, 5.71; N, 10.00 %
 $[\alpha]_D^{30}$: -2.3 (c, 3.3, MeOH).

(5) N-Benzoyl L-Alanyl-L-Serine Methyl Ester (Bz-Ala-Ser-OMe, 9):



(i) N-Benzoyl alanine (Bz-Ala-OH): (93%)

mp. : 151-152°C

(ii) Bz-Ala-Ser-OMe (9): (65%)

mp. : 135-136°C (lit.¹¹⁵)

ir : ν_{max} (KBr) cm^{-1} : 3455 (OH), 3325 (NH), 1740 (ester), 1655 (amide I), 1625 (amide I), 1600, 1570 (amide II), 1530 (amide II).

nmr : δ [$\text{CDCl}_3 + (\text{CD}_3)_2\text{SO}$]: 1.47 (3H, d, $J=7.5$ Hz, Ala CH_3), 3.75 (3H, s, COOCH_3), 3.87 (2H, m, Ser C^βH_2), 4.39-4.95 (2H, m, Ala $\text{C}^\alpha\text{H} + \text{Ser C}^\alpha\text{H}$), 7.20-8.00 (7H, m, Ala NH + Ser NH + aromatic protons).

anal: found: C, 57.42; H, 6.38; N, 9.71 %

Calc. for $C_{14}H_{18}N_2O_5$: C, 57.14; H, 6.12; N, 9.52 %
 $[\alpha]_D^{30}$: +10.8 (c, 0.4, MeOH).

(6) N-Benzoyl L-Leucyl-L-Serine Methyl Ester (Bz-Leu-Ser-OMe, 11):



(i) N-Benzoyl leucine (Bz-Leu-OH):

Step I: Bz-Cl (5.8 mL, 50 mmol) and 2N NaOH (25 mL) were simultaneously added to an ice-cooled and stirred suspension of L-Leucine (6.55g, 50 mmol) in 2N NaOH (25 mL). The addition of alkali was controlled to keep the medium basic throughout. After 30 min. of additional stirring, the reaction mixture was extracted with ether, the aqueous layer acidified with 2N HCl, extracted with ether, dried, admixed with cyclohexylamine (~10 mL), evaporated and the residue on crystallization from MeOH/Et₂O gave 11g (66%) of N-benzoyl leucine cyclohexylammonium salt (mp. 157-158°C) as white crystals.

Step II: A suspension of cyclohexylamine salt of Bz-Leu-OH (6.15g, 184 mmol) in EtOAc was mixed with 2N HCl (50 mL), shaken, layer separated, aq. portion extracted with EtOAc, dried and evaporated in vacuo and the residue on crystallization from EtOAc:hexane gave 4.18g (95%) of Bz-Leu-OH, mp. 106°C (lit.¹¹¹ mp. 106°C).

(ii) Bz-Leu-Ser-OMe (11): (70%)

mp. : $95-97^{\circ}\text{C}$

ir : ν_{max} (KBr) cm^{-1} : 3284 (NH), 1750 (ester), 1638 (amide I), 1544 (amide II).

anal: Found: C, 60.36; H, 7.42; N, 8.42 %

Calc. for $\text{C}_{17}\text{H}_{24}\text{N}_2\text{O}_5$: C, 60.71; H, 7.14; N, 8.33 %

$[\alpha]_{\text{D}}^{30}$: +24.1 (c, 3.3, MeOH).

(7) N-Benzoyl L-Phenylalanyl-L-Serine Methyl Ester (Bz-Phe-Ser-OMe, 13):

Bz-Phe-OH + H-Ser-OMe.HCl $\xrightarrow{\text{Method VI}}$ Bz-Phe-Ser-OMe (13)

(i) N-Benzoyl phenylalanine (Bz-Phe-OH): (95%)

mp. : $145-146^{\circ}\text{C}$ (lit.¹¹² mp. $145-146^{\circ}\text{C}$)

ir : ν_{max} (KBr) cm^{-1} : 3280 (NH), 1700 (acid), 1630 (amide I), 1515 (amide II).

(ii) Bz-Phe-Ser-OMe (13): (63%)

mp. : $105-106^{\circ}\text{C}$

ir : ν_{max} (KBr) cm^{-1} : 3330 (OH), 3280 (NH), 1740 (ester), 1725, 1635 (amide I), 1575 (amide II), 1545 (amide II), 1535 (amide II).

ms : m/z: 370 (M)⁺.

anal: Found: C, 65.07; H, 6.23; N, 7.73 %

Calc. for $\text{C}_{20}\text{H}_{22}\text{N}_2\text{O}_5$: C, 64.86; H, 5.94; N, 7.57 %

$[\alpha]_{\text{D}}^{30}$: +2.1 (c, 3.3, MeOH).

(8) N-Benzoyl L-Aspartyl-L-Serine Dimethyl Ester (Bz-Asp(β -OMe)-Ser-OMe, 15):

Bz-Asp(β -OMe)-OH + H-Ser-OMe.HCl $\xrightarrow{\text{Method VI}}$ Bz-Asp(β -OMe)-Ser-OMe (15)

(i) Aspartic acid β -methyl ester hydrochloride (Asp(β -OMe)-OH.HCl):

To an ice-cooled and stirred dry MeOH (25 mL), was added SOCl_2 (3.85 mL, 52.5 mmol) followed by L-Asp (5g, 37.5 mmol) in lots. The reaction mixture was allowed to attain room temperature, left stirred for 0.5h, admixed with dry Et_2O , filtered and dried to yield 5.32g (77%) of Asp(β -OMe)-OH.HCl, mp. 192-193°C (lit.¹¹³ mp. 190°C).

(ii) N-Benzoyl aspartic acid β -methyl ester (Bz-Asp(β -OMe)-OH):

To an ice-cooled and vigorously hand shaken solution of Asp(β -OMe)-OH.HCl (12g, 65.2 mmol) in satd. aq. NaHCO_3 (600 mL), was added, in drops, Bz-Cl (9 mL, 76.8 mmol), maintaining the medium basic throughout by addition of, in lots, satd. NaHCO_3 solution. The reaction mixture was left stirred for 2h, cooled, adjusted to pH~2 with 2N HCl (~130 mL), saturated with NaCl, extracted with Et_2O (3x100 mL), dried, solvents evaporated and the residue on crystallization from dry PhH gave 12.42g (76%) of Bz-Asp(β -OMe)-OH, mp. 126-127°C (lit.¹¹⁴ mp. 125-126°C).

ir : ν_{max} (KBr) cm^{-1} : 3320 (NH), 1750 (ester), 1720 (acid), 1630 (amide I), 1530 (amide II).

(iii) Bz-Asp(β -OMe)-Ser-OMe (15): (78%)

mp. : 135-136°C

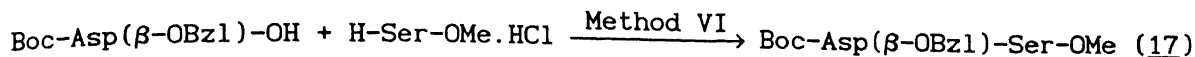
ir : ν_{max} (KBr) cm^{-1} : 3428 (OH), 3286 (NH), 1747 (ester), 1718, 1652 (amide I), 1562 (amide II), 1542 (amide II).

nmr : $\delta(\text{CDCl}_3)$: 2.94 (2H, dd, $J=5.5$ Hz, 1Hz, Asp C^βH_2), 3.72, 3.75 (3H, 3H, s, s, $\text{COOCH}_3 \times 2$), 3.91 (2H, d, $J=3$ Hz, Ser C^βH_2), 4.56 (1H, m, Ser C^αH), 5.00 (1H, m, Asp C^αH), 7.28-7.84 (7H, m, Asp NH + Ser NH + aromatic protons).

ms : m/z : 353 (MH)⁺.

anal: Found: C, 54.86; H, 5.90; N, 8.15 %

Calc. for $\text{C}_{16}\text{H}_{20}\text{N}_2\text{O}_7$: C, 54.54; H, 5.68; N, 7.95 %

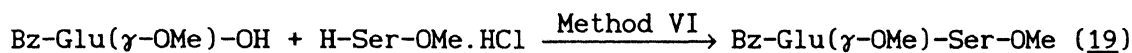
(9) N-^tButyloxycarbonyl β-O-Benzyl L-Aspartyl-L-Serine Methyl Ester(Boc-Asp(β-OBzl)-Ser-OMe, 17):Boc-Asp(β-OBzl)-Ser-OMe (17): (58%)

mp. : syrup

ir : ν_{max} (KBr) cm^{-1} : 3370 (br, NH, OH), 1740 (ester), 1670 (amide I), 1530 (amide II).

nmr : δ (60 MHz, CDCl_3): 1.43 (9H, s, Boc $\text{CH}_3 \times 3$), 2.83 (2H, d, $J=5.5$ Hz, Asp C^βH_2), 3.70 (3H, s, COOCH_3), 3.80 (2H, br, Ser C^βH_2) 4.53 (2H, m, Asp C^αH + Ser C^αH), 5.06 (2H, s, Bzl CH_2), 6.03 (1H, d, $J=8.7$ Hz, Asp NH), 7.23 (5H, s, aromatic protons), 7.46 (1H, d, $J=7.25$ Hz, Ser NH).

anal : Found: C, 56.47; H, 6.38; N, 6.87 %

Calc. for $\text{C}_{20}\text{H}_{28}\text{N}_2\text{O}_8$: C, 56.60; H, 6.60; N, 6.60 % $[\alpha]_{\text{D}}^{30}$: +25.53 (c, 0.32, CHCl_3).(10) N-Benzoyl L-Glutamyl-L-Serine Dimethyl Ester (Bz-Glu(γ-OMe)-Ser-OMe, 19):

(i) Glutamic acid γ-methyl ester (Glu(γ-OMe)-OH):

To an ice-cooled dry MeOH (120 mL), was added, in drops, SOCl_2 (7.2 mL, 100 mmol). The reaction mixture was allowed to attain room temperature, admixed with L-Glu (14.7g, 100 mmol), stirred at room temperature for 0.5h, cooled to 0-5°C, admixed with, over 5 min., Et_3N (35 mL, 250 mmol), filtered and dried to yield 13g (81%) of Glu(γ-OMe)-OH, mp. 183°C (lit.¹¹⁶ mp. 182°C).

(ii) N-Benzoyl glutamic acid γ-methyl ester (Bz-Glu(γ-OMe)-OH):

To an ice-cooled and stirred solution of Glu(γ-OMe)-OH (2.64g, 16.5 mmol) in satd. aq. NaHCO_3 (175 mL) was added, in drops, Bz-Cl (2.25 mL, 19.2 mmol), maintaining the medium basic throughout. The reaction mixture was left stirred at room temperature for 3h, cooled,

adjusted to pH~2 with 2N HCl (~60 mL), saturated with NaCl, extracted with EtOAc (3x25 mL), dried and evaporated to yield 2.89g (66%) of Bz-Glu(γ -OMe)-OH, mp. 106-107°C (dry acetone/hexane)(lit.¹¹⁷ mp. 107°C).

(iii) Bz-Glu(γ -OMe)-Ser-OMe (19): (65%)

mp. : 134-136°C

ir : ν_{\max} (KBr)cm⁻¹: 3273 (br, NH, OH), 1734 (ester), 1707, 1655 (amide I), 1626 (amide I), 1576 (amide II), 1532 (amide II).

nmr : δ (CDCl₃): 2.09-2.71 (4H, m, Glu C ^{β} H₂ + Glu C ^{α} H₂), 3.68, 3.76 (3H, 3H, s, s, COOCH₃x2), 4.00 (2H, m, Ser C ^{β} H₂), 4.68 (2H, m, Glu C ^{α} H + Ser C ^{α} H), 7.34-8.00 (7H, m, Glu NH + Ser NH + aromatic protons).

anal: Found: C, 55.64; H, 6.36; N, 7.33 %

Calc. for C₁₇H₂₂N₂O₇: C, 55.74; H, 6.01; N, 7.65 %

(11) N-Benzyloxycarbonyl L-Asparaginyl-L-Serine Methyl Ester:

(Z-Asn-Ser-OMe, 21):

Z-Asn-OH + H-Ser-OMe.HCl $\xrightarrow{\text{Method VIII}}$ Z-Asn-Ser-OMe (21)

(i) N-Benzyloxycarbonyl asparagine: (90%)

mp. : 183-184°C (lit.¹¹⁸ mp. 184-186°C)

(ii) Z-Asn-Ser-OMe (21): (65%)

mp. : 194-195°C (lit.¹¹⁹ mp. 197-199°C)

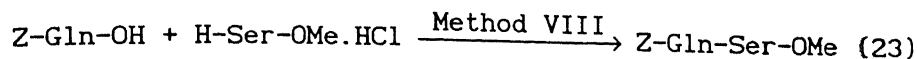
ir : ν_{\max} (KBr)cm⁻¹: 3421 (OH), 3298 (NH), 1731 (ester), 1686 (amide I), 1653 (amide I), 1609, 1539 (amide II).

nmr : δ [CDCl₃ + (CD₃)₂SO]: 2.50 (2H, d, J=5 Hz, Asn C ^{β} H₂), 3.68 (5H, s+m, COOCH₃ + Ser C ^{β} H₂), 4.45 (2H, m, Asn C ^{α} H + Ser C ^{α} H), 5.09 (2H, s, Z CH₂), 6.90 (1H, br, Asn NH), 7.34 (7H, brs, Asn CONH₂ + aromatic protons), 8.09 (1H, d, J=8.75 Hz, Ser NH).

anal: Found: C, 52.43; H, 5.63; N, 11.62 %

Calc. for C₁₆H₂₁N₃O₇: C, 52.32; H, 5.72; N, 11.44 %

(12) N-Benzylloxycarbonyl L-Glutaminyl-L-Serine Methyl Ester (Z-Gln-Ser-OMe,

23):

(i) N-Benzylloxycarbonyl glutamine (Z-Gln-OH): (92%)

mp. : 130-131°C (lit.¹²⁰ mp. 130-131°C)(ii) Z-Gln-Ser-OMe (23): (63%)mp. : 156-160°C (lit.¹²¹ mp. 156-160°C)

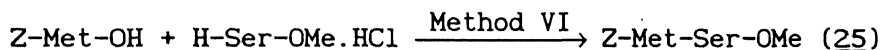
ir : ν_{max} (KBr) cm^{-1} : 3403 (OH), 3312 (NH), 1747 (ester), 1642 (amide I), 1535 (amide II).

nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 1.68-2.40 (4H, brm, Gln C^βH_2 + Gln $\text{C}^\gamma\text{H}_2$), 3.68 (5H, brs, COOCH_3 + Ser C^βH_2), 4.00-4.59 (2H, m, Gln C^αH + Ser C^αH), 5.06 (2H, s, Z CH_2), 6.50 (1H, br, Gln NH), 6.84-7.56 (7H, s + br, Gln CONH_2 + aromatic protons), 8.03 (1H, br, Ser NH).

anal: Found: C, 53.93; H, 6.18; N, 11.44 %

Calc. for $\text{C}_{17}\text{H}_{23}\text{N}_3\text{O}_7$: C, 53.54; H, 6.04; N, 11.02 %

(13) N-Benzylloxycarbonyl L-Methionyl-L-Serine Methyl Ester (Z-Met-Ser-OMe,

25):

(i) N-Benzylloxycarbonyl methionine (Z-Met-OH): (95%)

mp. : 68-69°C (lit.¹²² mp. 69-70°C)(ii) Z-Met-Ser-OMe (25): (90%)

mp. : 143-144°C

ir : ν_{max} (KBr) cm^{-1} : 3535 (OH), 3300 (NH), 1725 (ester), 1682 (amide I), 1645 (amide I), 1555 (amide II), 1540 (amide II).

nmr : δ (CDCl_3): 2.09 (5H, s + m, Met C^βH_2 + Met S- CH_3), 2.56 (2H, t, Met $\text{C}^\gamma\text{H}_2$), 3.75 (3H, s, COOCH_3), 3.87 (2H, brd, Ser C^βH_2), 4.15-4.75 (2H, m, Met C^αH + Ser C^αH), 5.06 (2H, s, Z CH_2), 5.62 (1H, d, $J=7.5$ Hz, Met NH), 6.90-7.53 (6H, s + m, Ser NH + aromatic protons).

anal: Found: C, 53.40; H, 6.38; N, 7.26 %

Calc. for $C_{17}H_{24}N_2O_6S$: C, 53.12; H, 6.25; N, 7.29 %

$[\alpha]_D^{30}$: +20.94 (c, 0.42, $CHCl_3$).

(14) N-Benzoyl L-Valyl-L-Serine Methyl Ester (Bz-Val-Ser-OMe, 27):

Bz-Val-OH + H-Ser-OMe.HCl $\xrightarrow{\text{Method VI}}$ Bz-Val-Ser-OMe (27)

Bz-Val-Ser-OMe (27): (78%)

mp. : 169-170°C

ir : ν_{\max} (KBr) cm^{-1} : 3340 (OH), 3290 (NH), 1750 (ester), 1623 (amide I), 1570 (amide II).

nmr : δ ($CDCl_3$): 1.06 (6H, d, $J=5.0$ Hz, Val $CH_3 \times 2$), 2.18 (1H, m, Val $C^\beta H$), 3.65-4.10 (5H, s + m, $COOCH_3$ + Ser $C^\beta H_2$), 4.62 (2H, m, Val $C^\alpha H$ + Ser $C^\alpha H$), 6.93-8.00 (7H, m, Val NH + Ser NH + aromatic protons).

anal: Found: C, 59.86; H, 6.47; N, 8.58 %

Calc. for $C_{16}H_{22}N_2O_5$: C, 59.63; H, 6.83; N, 8.70 %

$[\alpha]_D^{30}$: +15.44 (c, 1.58, $CHCl_3$).

(15) N-Benzoyl L-Prolyl-L-Serine Methyl Ester (Bz-Pro-Ser-OMe, 29):

Bz-Pro-OH + H-Ser-OMe.HCl $\xrightarrow{\text{Method VI}}$ Bz-Pro-Ser-OMe (29)

(i) N-Benzoyl proline (Bz-Pro-OH): (90%)

mp. : 153-154°C (lit.¹²³ mp. 153-154°C)

ir : ν_{\max} (KBr) cm^{-1} : 3330 (NH), 1710 (COOH).

(ii) Bz-Pro-Ser-OMe (29): (40%)

mp. : 71-72°C

ir : ν_{\max} (KBr) cm^{-1} : 3460 (OH), 3390 (NH), 3320 (NH), 1743 (ester), 1642 (amide I), 1613 (amide I), 1570 (amide II), 1555 (amide II).

nmr : δ ($CDCl_3$): 2.18 (4H, m, Pro $C^\beta H_2$ + Pro $C^\gamma H_2$), 3.46-4.12 (7H, s + m, $COOCH_3$ + Pro $C^\delta H_2$ + Ser $C^\beta H_2$), 4.62 (2H, m, Pro $C^\alpha H$ + Ser $C^\alpha H$), 7.03-7.81 (6H, m, Ser NH + aromatic protons).

anal: Found: C, 59.68; H, 6.52; N, 8.57 %

Calc. for $C_{16}H_{20}N_2O_5$: C, 60.00; H, 6.25; N, 8.75 %

$[\alpha]_D^{30}$: -26.50 (c, 0.8, $CHCl_3$).

(16) N-^tButyloxycarbonyl L-Arginyl(N^GNO_2)-L-Serine Methyl Ester

(Boc-Arg(N^GNO_2)-Ser-OMe, 31):

Boc-Arg(N^GNO_2)-OH + H-Ser-OMe.HCl $\xrightarrow{\text{Method VI}}$ Boc-Arg(N^GNO_2)-Ser-OMe (31)

Boc-Arg(N^GNO_2)-Ser-OMe (31): (52%)

mp. : 74°C

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3318 (br, OH, NH), 1744 (ester), 1661 (amide I), 1600, 1532 (br, amide II, NO_2), 1355 (NO_2).

nmr : δ [$CDCl_3$ + $(CD_3)_2SO$] : 1.44 (9H, s, Boc CH_3 x3), 1.72 (4H, m, Arg $C^\beta H_2$ + Arg $C^\gamma H_2$), 3.34 (2H, m, Arg $C^\delta H_2$), 3.72-4.00 (5H, s + m, $COOCH_3$ + Ser $C^\beta H_2$), 4.12 (1H, m, Arg $C^\alpha H$), 4.52 (1H, m, Ser $C^\alpha H$), 6.31 (1H, d, $J=7.5$ Hz, Arg NH), 7.56-8.18 (4H, m, Ser NH + Guanidinium NH x3).

ms : m/z: 421 (MH)⁺.

anal: Found: C, 43.25; H, 6.80; N, 19.64 %

Calc. for $C_{15}H_{28}N_6O_8$: C, 42.85; H, 6.66; N, 20.00 %

(17) N-Benzoyl L-Prolyl-L-Phenylalanyl-L-Serine Methyl Ester

(Bz-Pro-Phe-Ser-OMe, 33):

(a) Bz-Pro-OH + H-Phe-OMe.HCl $\xrightarrow{\text{Method VI}}$ Bz-Pro-Phe-OMe

(i) Phenylalanine methyl ester hydrochloride (Phe-OMe.HCl): (89%)

mp. : 161°C (lit.¹²⁴ mp. 160°C)

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 1740 (ester).

(ii) N-Benzoyl L-prolyl-L-phenylalanine methyl ester (Bz-Pro-Phe-OMe):

mp. : 117-118°C

(b) Bz-Pro-Phe-OMe $\xrightarrow{\text{Method IX}}$ Bz-Pro-Phe-OH

Bz-Pro-Phe-OH : (81%)

mp. : 188-190°C

(c) Bz-pro-Phe-OH + H-Ser-OMe.HCl $\xrightarrow{\text{Method VI}}$ Bz-Pro-Phe-Ser-OMe (33)

Bz-Pro-Phe-Ser-OMe (33): (68%)

mp. : 182-184°C

ir : ν_{\max} (KBr) cm^{-1} : 3400 (OH), 3340 (NH), 1742 (ester), 1660 (amide I), 1600, 1570 (amide II), 1535 (amide II).

nmr : δ (CDCl_3): 1.96 (4H, m, Pro C^βH_2 + Pro $\text{C}^\gamma\text{H}_2$), 3.15-3.93 (9H, s + m, COOCH_3 + Pro $\text{C}^\delta\text{H}_2$ + Phe C^βH_2 + Ser C^βH_2), 4.50 (3H, m, Pro C^αH + Phe C^αH + Ser C^αH), 6.90 (1H, d, $J=7.5$ Hz, Phe NH), 7.15-7.59 (11H, s + m, Ser NH + aromatic protons).

anal: Found: C, 64.21; H, 6.32; N, 8.72 %

Calc. for $\text{C}_{25}\text{H}_{29}\text{N}_3\text{O}_6$: C, 64.24; H, 6.21; N, 8.99 %

$[\alpha]_D^{30}$: -112.3 (c, 3.4, CHCl_3).

(18) N-^tButyloxycarbonyl L-Alanyl-L-Alanyl-L-Serine Methyl Ester

(Boc-Ala-Ala-Ser-OMe, 36):

(a) N-^tButyloxycarbonyl L-alanyl-L-alanine methyl ester

(Boc-Ala-Ala-OMe, 35):

Boc-Ala-OH + H-Ala-OMe.HCl $\xrightarrow{\text{Method VI}}$ Boc-Ala-Ala-OMe (35)

(i) N-^tButyloxycarbonyl alanine (Boc-Ala-OH): (80%)

mp. : 78-79°C (lit.¹²⁵ mp. 73-74°C)

(ii) Alanine methyl ester hydrochloride (Ala-OMe.HCl): (90%)

mp. : 110-111°C (lit.¹²⁶ mp. 110-111°C)

(iii) Boc-Ala-Ala-OMe (35): (82%)

mp. : 98-99°C (lit.¹²⁷)

ir : ν_{\max} (KBr) cm^{-1} : 3260 (br, NH), 1725 (ester), 1640 (br, amide I), 1525 (br, amide II).

(b) Boc-Ala-Ala-OMe $\xrightarrow{\text{Method IX}}$ Boc-Ala-Ala-OH

Boc-Ala-Ala-OH : (85%)

mp. : 88-89°C

(c) Boc-Ala-Ala-OH + H-Ser-OMe.HCl

$\xrightarrow{\text{Method VI}}$ Boc-Ala-Ala-Ser-OMe (36)

Boc-Ala-Ala-Ser-OMe (36): (68%)

mp. : 156-158°C

nmr : δ (CDCl₃): 1.43 (15H, s + m, Boc CH₃x3 + Ala CH₃x2), 3.81 (3H, s, COOCH₃), 3.96 (2H, m, Ser C ^{β} H₂), 4.18 (1H, m, Ser C ^{α} H), 4.62 (2H, m, Ala C ^{α} Hx2), 5.28 (1H, d, J=7.5 Hz, Ala NH(Boc)), 7.06 (1H, d, J=7.5 Hz, NH), 7.46 (1H, d, J=7.5 Hz, NH).

anal: Found: C, 49.58; H, 7.33; N, 11.52 %

Calc. for C₁₅H₂₇N₃O₇: C, 49.86; H, 7.48; N, 11.63 %

$[\alpha]_D^{30}$: -24.00 (c, 0.5, CHCl₃).

(19) N-Benzoyl L-Valyl-L-Phenylalanyl-L-Serine Methyl Ester

(Bz-Val-Phe-Ser-OMe, 38):

(i) Bz-Val-OH + H-Phe-OMe.HCl $\xrightarrow{\text{Method VI}}$ Bz-Val-Phe-OMe

Bz-Val-Phe-OMe : (83%)

mp. : 146-148°C

ir : ν_{max} (KBr)cm⁻¹: 3315 (NH), 1720 (ester), 1702, 1655 (amide I), 1630 (amide I), 1595, 1563 (amide II), 1522 (amide II).

(ii) Bz-Val-Phe-OMe $\xrightarrow{\text{Method IX}}$ Bz-Val-Phe-OH

Bz-Val-Phe-OH : (78%)

mp. : 185-187°C

(iii) Bz-Val-Phe-OH + H-Ser-OMe.HCl $\xrightarrow{\text{Method VI}}$ Bz-Val-Phe-Ser-OMe (38)

Bz-Val-Phe-Ser-OMe (38) : (87%)

mp. : 165-167°C

ir : ν_{max} (KBr)cm⁻¹: 3338 (NH), 3298 (NH), 1743 (ester), 1630 (amide I), 1580, 1538 (amide II).

anal: Found: C, 64.34; H, 6.76; N, 8.79 %

Calc. for C₂₅H₃₁N₃O₆: C, 63.96; H, 6.61; N, 8.95 %

$[\alpha]_D^{30}$: -13.9 (c, 3.3, MeOH).

(20) N-Benzoyl L-Threonine Methyl Ester (Bz-Thr-OMe, 40):

(1) Benzoyl threonine (Bz-Thr-OH): (60%)

mp. : 134-135°C (lit.¹²⁸ mp. 143-144°C)

(11) Bz-Thr-OMe (40):

To a solution of Bz-Thr-OH (1g, 4.5 mmol) in MeOH (20 mL) was added an excess of ice-cold ethereal solution of CH_2N_2 . The reaction mixture was evaporated and the residue on crystallization from EtOAc/hexane gave 0.84g (79%) of Bz-Thr-OMe.

mp. : 91-92°C (lit.¹²⁹ mp. 96°C)

ir : ν_{max} (KBr) cm^{-1} : 3410 (OH), 3345 (NH), 1730 (ester), 1630 (amide I), 1510 (amide II).

(21) N-Benzoyl Glycyl-L-Threonine Methyl Ester (Bz-Gly-Thr-OMe, 41):

Bz-Gly-OH + H-Thr-OMe.HCl $\xrightarrow{\text{Method VI}}$ Bz-Gly-Thr-OMe (41)

(i) Threonine methyl ester hydrochloride (Thr-OMe.HCl): (93%)

mp. : thick syrupy liquid

(ii) Bz-Gly-Thr-OMe (41): (90%)

mp. : 138-140°C

ir : ν_{max} (KBr) cm^{-1} : 3485 (OH), 3370 (NH), 3315 (NH), 1723 (ester), 1671 (amide I), 1647 (amide I), 1580 (amide II), 1549 (amide II).

nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 1.15 (3H, d, $J=6.5$ Hz, Thr CH_3), 3.71 (3H, s, COOCH_3), 4.12 (2H, d, $J=5.0$ Hz, Gly CH_2), 4.25 (1H, m, Thr C^βH), 4.46 (1H, dd, $J=8.75$ Hz, 2.5 Hz Thr C^αH), 7.28-8.06 (6H, m, Gly NH + aromatic protons), 8.37 (1H, m, Thr NH).

anal: Found: C, 57.40; H, 6.26; N, 9.83 %

Calc. for $\text{C}_{14}\text{H}_{18}\text{N}_2\text{O}_5$: C, 57.14; H, 6.12; N, 9.52 %

$[\alpha]_{\text{D}}^{30}$: -6.21 (c, 3.3, MeOH).

(22) N-Benzoyl L-Alanyl-L-Threonine Methyl Ester (Bz-Ala-Thr-OMe, 42):

Bz-Ala-OH + H-Thr-OMe.HCl $\xrightarrow{\text{Method VI}}$ Bz-Ala-Thr-OMe (42)

Bz-Ala-Thr-OMe (42): (64%)

mp. : 68-70°C (lit.¹³⁰)

ir : ν_{\max} (KBr) cm^{-1} : 3320 (NH), 1740 (ester), 1660 (amide I), 1530 (amide II), 1490.

nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 1.15 (3H, d, $J=6.5$ Hz, Thr CH_3), 1.53 (3H, d, $J=6.5$ Hz, Ala CH_3), 3.78 (3H, s, COOCH_3), 4.28 (1H, m, Thr C^βH), 4.56 (1H, dd, $J=8.75$ Hz, 2.5 Hz, Thr C^αH), 4.88 (1H, m, Ala C^αH), 7.19–8.00 (7H, m, Ala NH + Thr NH + aromatic protons).

ms : m/z : 309 (MH) $^+$.

anal: Found: C, 57.73; H, 6.82; N, 8.49 %

Calc. for $\text{C}_{15}\text{H}_{20}\text{N}_2\text{O}_5$: C, 58.44; H, 6.49; N, 9.09 %

$[\alpha]_D^{30}$: +6.1 (c, 3.3, CHCl_3).

(23) N-Benzoyl L-Leucyl-L-Threonine Methyl Ester (Bz-Leu-Thr-OMe, 43):

Bz-Leu-OH + H-Thr-OMe.HCl $\xrightarrow{\text{Method VI}}$ Bz-Leu-Thr-OMe (43)

Bz-Leu-Thr-OMe (43): (65%)

mp. : 113–114 $^\circ\text{C}$

ir : ν_{\max} (KBr) cm^{-1} : 3300 (NH), 1747 (ester), 1670 (amide I), 1639 (amide I), 1537 (amide II).

nmr : δ (CDCl_3): 0.94 (6H, brs, Leu $\text{CH}_3 \times 2$), 1.20 (3H, d, $J=6.5$ Hz, Thr CH_3), 1.73 (3H, br, Leu C^βH_2 + Leu C^γH), 3.76 (3H, s, COOCH_3), 4.00 (1H, br, Thr OH), 4.31 (1H, br, Thr C^βH), 4.56 (1H, m, Thr C^αH), 4.85 (1H, m, Leu C^αH), 7.05–8.14 (7H, m, Leu NH + Thr NH + aromatic protons).

ms : m/z : 351 (MH) $^+$.

anal: Found: C, 61.23; H, 7.18; N, 8.22 %

Calc. for $\text{C}_{18}\text{H}_{26}\text{N}_2\text{O}_5$: C, 61.71; H, 7.43; N, 8.00 %

$[\alpha]_D^{30}$: -5.4 (c, 3.3, MeOH).

(24) N-Benzoyl L-Phenylalanyl-L-Threonine Methyl Ester (Bz-Phe-Thr-OMe,

44):

Bz-Phe-OH + H-Thr-OMe.HCl $\xrightarrow{\text{Method VI}}$ Bz-Phe-Thr-OMe (44)

Bz-Phe-Thr-OMe (44): (63%)

mp. : 145-146°C

ir : ν_{\max} (KBr) cm^{-1} : 3475 (OH), 3310 (NH), 3270 (NH), 1720 (ester), 1671 (amide I), 1641 (amide I), 1541 (amide II).

ms : m/z: 384 (M)⁺.

anal: Found: C, 65.69; H, 6.37; N, 7.18 %

Calc. for $\text{C}_{21}\text{H}_{24}\text{N}_2\text{O}_5$: C, 65.62; H, 6.25; N, 7.29 %

(25) N-Benzoyl L-Glycyl-L-Phenylalanyl-L-Threonine Methyl Ester

(Bz-Gly-Phe-Thr-OMe, 45):

(i) Bz-Gly-OH + H-Phe-OMe.HCl $\xrightarrow{\text{Method VI}}$ Bz-Gly-Phe-OMe

Bz-Gly-Phe-OMe : (86%)

mp. : 105-106°C

ir : ν_{\max} (KBr) cm^{-1} : 3310, 1745 (ester), 1660 (amide I), 1630 (amide I), 1595, 1570 (amide II), 1535 (br, amide II).

(ii) Bz-Gly-Phe-OMe $\xrightarrow{\text{Method IX}}$ Bz-Gly-Phe-OH

Bz-Gly-Phe-OH: (79%)

mp. : gummy foam

(iii) Bz-Gly-Phe-OH + H-Thr-OMe.HCl $\xrightarrow{\text{Method VI}}$ Bz-Gly-Phe-Thr-OMe (45)

Bz-Gly-Phe-Thr-OMe (45): (79%)

mp. : 157-159°C

ir : ν_{\max} (KBr) cm^{-1} : 3320 (NH), 1750 (ester), 1660 (amide I), 1555 (amide II), 1530 (amide II).

nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 1.14 (3H, d, J=6.5 Hz, Thr CH_3), 3.12 (2H, m, Phe C^βH_2), 3.70 (3H, s, COOCH_3), 3.96 (2H, dd, J=5.0 Hz, 1.0 Hz, Gly CH_2), 4.19-4.60 (2H, m, Thr C^βH + Thr C^αH), 4.75 (1H, m, Phe C^αH), 7.00-8.00 (12H, m, Phe NH + Thr NH + aromatic protons), 8.15 (1H, t, Gly NH).

ms : m/z: 441 (M)⁺.

anal: Found: C, 62.38; H, 6.29; N, 9.17 %

Calc. for $\text{C}_{23}\text{H}_{27}\text{N}_3\text{O}_6$: C, 62.58; H, 6.12; N, 9.52 %

(26) N-Benzoyl L-Valyl-L-Phenylalanyl-L-Threonine Methyl Ester

(Bz-Val-Phe-Thr-OMe, 47):Bz-Val-Phe-OH + H-Thr-OMe.HCl $\xrightarrow{\text{Method VI}}$ Bz-Val-Phe-Thr-OMe (47)Bz-Val-Phe-Thr-OMe (47): (52%)

mp. : 205-207°C

ir : ν_{max} (KBr) cm^{-1} : 3495 (OH), 3340 (NH), 3320 (NH), 3280 (NH),
1722 (ester), 1630 (amide I), 1580, 1540 (amide II).

nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 0.75-1.34 (9H, m, Thr CH_3 + Val $\text{CH}_3 \times 2$), 2.14 (1H, m, Val C^βH), 3.15 (2H, t, Phe C^βH_2), 3.75 (3H, s, COOCH_3), 4.18-5.15 (4H, m, Val C^αH + Phe C^αH + Thr C^αH + Thr C^βH), 7.09-8.00 (13H, m, Val NH + Phe NH + Thr NH + aromatic protons).

anal: Found: C, 64.65; H, 6.67; N, 8.56 %

Calc. for $\text{C}_{26}\text{H}_{33}\text{N}_3\text{O}_6$: C, 64.60; H, 6.83; N, 8.69 % $[\alpha]_D^{30}$: -20.9 (c, 2.3, MeOH).(27) N-^tButyloxycarbonyl L-Alanyl-L-Alanyl-L-Threonine Methyl Ester(Boc-Ala-Ala-Thr-OMe, 48):Boc-Ala-Ala-OH + H-Thr-OMe.HCl $\xrightarrow{\text{Method VI}}$ Boc-Ala-Ala-Thr-OMe (48)Boc-Ala-Ala-Thr-OMe (48): (53%)

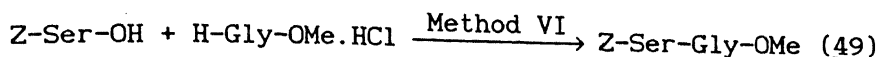
mp. : 155-156°C

ir : ν_{max} (KBr) cm^{-1} : 3390 (OH), 3310 (NH), 1740 (ester), 1695
(carbamate), 1638 (amide I), 1530 (amide II).

nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 1.03-1.60 (15H, m, Thr CH_3 + Ala $\text{CH}_3 \times 2$ + Boc $\text{CH}_3 \times 3$), 3.81 (3H, s, COOCH_3), 4.06-4.81 (4H, m, Ala $\text{C}^\alpha\text{H} \times 2$ + Thr C^αH + Thr C^βH), 5.65 (1H, d, $J=7.5$ Hz, Ala NH(Boc)), 7.46 (2H, m, Ala NH + Thr NH).

anal: Found: C, 50.87; H, 7.48; N, 11.06 %

Calc. for $\text{C}_{16}\text{H}_{29}\text{N}_3\text{O}_7$: C, 51.20; H, 7.73; N, 11.20 % $[\alpha]_D^{30}$: -53.9 (c, 3.3, MeOH).

(28) N-Benzylloxycarbonyl L-Seryl-Glycine Methyl Ester (Z-Ser-Gly-OMe, 49):

(i) N-Benzylloxycarbonyl serine (Z-Ser-OH): (94%)

mp. : 115-116°C (lit.¹³¹ mp. 119°C)

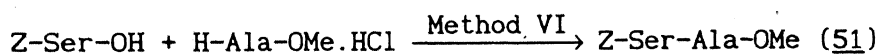
(ii) Glycine methyl ester hydrochloride (Gly-OMe.HCl): (96%)

mp. : 175-176°C (lit.¹³² mp. 175°C)(iii) Z-Ser-Gly-OMe (49): (78%)mp. : 98-99°C (lit.¹³³ mp. 105-106°C)

ir : ν_{max} (KBr) cm^{-1} : 3310 (NH), 1753 (ester), 1682 (amide I),
1648 (amide I), 1529 (amide II).

nmr : δ (CDCl_3): 3.71 (5H, s + m, COOCH_3 + Ser C^βH_2), 4.00 (2H, d, $J=6.25$ Hz, Gly CH_2), 4.26 (1H, m, Ser C^αH), 5.12 (2H, s, Z CH_2), 5.96 (1H, d, $J=7.5$ Hz, Ser NH), 6.96-7.46 (6H, s + m, Gly NH + aromatic protons).

anal: Found: C, 54.23; H, 5.55; N, 9.25 %

Calc. for $\text{C}_{14}\text{H}_{18}\text{N}_2\text{O}_6$: C, 54.19; H, 5.81; N, 9.03 %[α]_D²⁵: -8.1 (c, 3.3, CHCl_3).(29) N-Benzylloxycarbonyl L-Seryl-L-Alanine Methyl Ester (Z-Ser-Ala-OMe, 51):Z-Ser-Ala-OMe (51): (78%)mp. : 104-106°C (lit.¹³⁴ mp. 113-114°C)

ir : ν_{max} (KBr) cm^{-1} : 3314 (NH), 1762 (ester), 1694 (carbamate),
1657 (amide I), 1539 (amide II).

nmr : δ (CDCl_3): 1.33 (3H, d, $J=7.5$ Hz, Ala CH_3), 3.71 (5H, s+m, COOCH_3 + Ser C^βH_2), 4.15-4.75 (2H, m, Ala C^αH + Ser C^αH),
5.09 (2H, s, Z CH_2), 6.03 (1H, d, $J=7.5$ Hz, Ser NH), 7.36 (6H, s + m, Ala NH + aromatic protons).

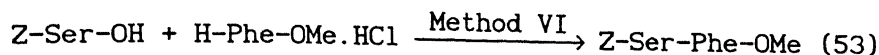
ms : m/z: 325 (MH)⁺.

anal: Found: C, 55.43; H, 6.37; N, 8.59 %

Calc. for $C_{15}H_{20}N_2O_6$: C, 55.55; H, 6.17; N, 8.64 %
 $[\alpha]_D^{25}$: -7.8 (c, 3.7, $CHCl_3$).

(30) N-Benzylloxycarbonyl L-Seryl-L-Phenylalanine Methyl Ester

(Z-Ser-Phe-OMe, 53):



Z-Ser-Phe-OMe (53): (50%)

mp. : 102-104°C (lit.¹³⁵ mp. 79-80°C)

ir : ν_{\max} (KBr) cm^{-1} : 3300 (NH), 1732 (ester), 1688 (carbamate),
 1650 (amide I), 1528 (amide II), 1450.

nmr : δ ($CDCl_3$): 3.02 (2H, brd, Phe $C^\beta H_2$), 3.61 (5H, s+m, $COOCH_3$
 + Ser $C^\beta H_2$), 4.22 (1H, m, Ser $C^\alpha H$), 4.79 (1H, m, Phe $C^\alpha H$),
 5.03 (2H, s, Z CH_2), 6.05 (1H, d, $J=7.5$ Hz, Ser NH),
 6.87-7.43 (11H, s+m, Phe NH + aromatic protons).

anal: Found: C, 62.74; H, 6.11; N, 7.26 %

Calc. for $C_{21}H_{24}N_2O_6$: C, 63.00; H, 6.00; N, 7.00 %
 $[\alpha]_D^{25}$: -2.7 (c, 3.3, MeOH).

(31) N-Benzylloxycarbonyl L-Seryl-L-Leucine Methyl Ester (Z-Ser-Leu-OMe, 55):



(i) Leucine methyl ester hydrochloride (Leu-OMe.HCl): (92%)

mp. : 149-150°C (lit.¹³⁶ mp. 151°C)

ir : ν_{\max} (KBr) cm^{-1} : 3205 (NH), 1740 (ester).

(ii) Z-Ser-Leu-OMe (55): (84%)

mp. : 77-78°C (lit.¹³⁷ mp. 73-74.5°C)

ir : ν_{\max} (KBr) cm^{-1} : 3400 (OH), 3310 (NH), 1745 (ester), 1695
 (carbamate), 1660 (amide I), 1645 (amide I), 1550 (amide
 II).

nmr : δ ($CDCl_3$): 0.84 (6H, d, $J=5.0$ Hz, Leu $CH_3 \times 2$), 1.53 (3H, m,
 Leu $C^\beta H_2$ + Leu $C^\gamma H$), 3.71 (5H, s + m, $COOCH_3$ + Ser $C^\beta H_2$),
 4.00-4.62 (2H, m, Leu $C^\alpha H$ + Ser $C^\alpha H$), 5.10 (2H, s, Z CH_2),

5.90 (1H, d, J=7.5 Hz, Ser NH), 7.03 (1H, d, J=7.5 Hz, Leu NH), 7.37 (5H, s, aromatic protons).

anal: Found: C, 59.33; H, 7.18; N, 7.43 %

Calc. for $C_{18}H_{26}N_2O_6$: C, 59.02; H, 7.10; N, 7.65 %

$[\alpha]_D^{25}$: -32.65 (c, 1.66, MeOH).

(32) N-Benzyloxycarbonyl L-Seryl-Anthranilic Acid Methyl Ester

(Z-Ser-Methylanthranilate, 57):

Z-Ser-OH + Methylanthranilate $\xrightarrow{\text{Method VI}}$ Z-Ser-Methylanthranilate (57)

Z-Ser-Methylanthranilate (57): (43%)

mp. : 100-101°C

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3459 (OH), 3391 (NH), 3293 (NH), 1708 (ester), 1680 (carbamate), 1626 (amide I), 1606, 1588, 1519 (amide II).

nmr : $\delta(\text{CDCl}_3)$: 3.90 (3H, s, COOCH_3), 4.16 (2H, m, Ser C^βH_2), 4.47 (1H, m, Ser C^αH), 5.20 (2H, s, Z CH_2), 5.84 (1H, brd, Ser NH), 7.03-7.66 (8H, m, aromatic protons + anthranilic NH + anthranilic ring protonx2), 8.06, 8.69 (1H, 1H, dd, dd, J=7.5 Hz, 1.25 Hz, anthranilic ring protons).

anal: Found: C, 60.82; H, 4.86; N, 7.78 %

Calc. for $C_{19}H_{20}N_2O_6$: C, 61.29; H, 5.38; N, 7.53 %

(33) N-Benzyloxycarbonyl L-Seryl-L-Tyrosine Methyl Ester (Z-Ser-Tyr-OMe, 59):

Z-Ser-OH + H-Tyr-OMe.HCl $\xrightarrow{\text{Method VI}}$ Z-Ser-Tyr-OMe (59)

(i) Tyrosine methyl ester hydrochloride (Tyr-OMe.HCl): (80%)

mp. : 188-189°C (lit.¹³⁸ mp. 190°C)

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3330 (NH), 1730 (ester).

(ii) Z-Ser-Tyr-OMe (59): (60%)

mp. : 105-106°C (lit.¹³⁹ mp. 115-116°C)

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3397 (OH), 3315 (NH), 1750 (ester), 1708, (carbamate), 1649 (amide I), 1570, 1515 (amide II), 1453.

nmr : δ (CDCl_3): 3.00 (2H, m, Tyr C^βH_2), 3.71 (5H, s+m, COOCH_3 + Ser C^βH_2), 4.20 (1H, m, Ser C^αH), 4.60 (1H, m, Tyr C^αH), 5.06 (2H, s, Z CH_2), 5.84 (1H, d, $J=7.5$ Hz, Ser NH), 6.50 (1H, brd, Tyr NH), 6.66-7.12 (4H, dd, Tyr ring protons), 7.31 (5H, s, aromatic protons).

anal: Found: C, 60.36; H, 5.68; N, 6.48 %

Calc. for $\text{C}_{21}\text{H}_{24}\text{N}_2\text{O}_7$: C, 60.58; H, 5.77; N, 6.73 %

$[\alpha]_D^{25}$: +3.66 (c, 3.33, MeOH).

(34) **N-Benzoyloxycarbonyl L-Seryl-L-Tryptophan Methyl Ester (Z-Ser-Trp-OMe, 61):**

Z-Ser-OH + H-Trp-OMe.HCl $\xrightarrow{\text{Method VI}}$ Z-Ser-Trp-OMe (61)

(i) Tryptophan methyl ester hydrochloride (Trp-OMe.HCl):

To ice-salt cooled (-10°C) and stirred dry MeOH (25 mL) was added, in drops, SOCl_2 (1.9 mL, 23.6 mmol) followed by, rapidly, L-Trp (2g, 12.5 mmol). From the resulting clear solution, after a short while, a solid precipitated. The reaction mixture was left stirred for an additional 4h at -5 to 0°C , allowed to attain room temperature, when the precipitated solid redissolved. The resulting clear yellow solution was left stirred at room temperature overnight, concentrated in vacuo to ~5mL, admixed with Et_2O , the mixture refrigerated for 4h, filtered, washed with dry ether and dried to yield TrpOMe.HCl (3g, 95%).

mp. : $214-215^\circ\text{C}$ (lit.¹⁴⁰ mp. $212.5-214^\circ\text{C}$)

ir : ν_{max} (KBr) cm^{-1} : 3270 (NH), 1740 (ester).

(ii) Z-Ser-Trp-OMe (61): (71%)

mp. : foamy solid (lit.¹⁴¹ mp. $101.5-103.5^\circ\text{C}$)

ir : ν_{max} (KBr) cm^{-1} : 3350 (NH), 1720 (ester), 1655 (amide I), 1508 (amide II), 1450.

nmr : δ (CDCl_3): 3.26 (2H, d, $J=5.0$ Hz, Trp C^βH_2), 3.71 (5H, s+m, COOCH_3 + Ser C^βH_2), 4.25 (1H, m, Ser C^αH), 4.81-5.25

(3H, s+m, Trp C $^{\alpha}$ H + Z CH $_2$), 6.03 (1H, d, J=7.5 Hz, Ser NH), 6.89-7.57 (11H, s+m, Trp NH + aromatic protons), 8.64 (1H, brs, Indole NH).

ms : m/z: 439 (M) $^{+}$.

anal: Found: C, 62.86; H, 5.34; N, 9.65 %

Calc. for C $_{23}$ H $_{25}$ N $_3$ O $_6$: C, 62.87; H, 5.69; N, 9.57 %

$[\alpha]_D^{25}$: +9.38 (c, 0.81, MeOH).

(35) N-Benzylloxycarbonyl L-Seryl-L-Proline Methyl Ester (Z-Ser-Pro-OMe, 62):

Z-Ser-OH + H-Pro-OMe HCl $\xrightarrow{\text{Method VI}}$ Z-Ser-Pro-OMe (62)

(i) Proline methyl ester hydrochloride (Pro-OMe.HCl): (90%)

mp. : oil (lit. 142 mp. oil)

(ii) Z-Ser-Pro-OMe (62): (40%)

mp. : 113-115 (lit. 143 mp. 117-121 $^{\circ}$ C)

ir : ν_{max} (KBr)cm $^{-1}$: 3396 (OH), 3280 (NH), 1735 (ester), 1713, (carbamate), 1617 (amide I), 1558 (amide II), 1531 (amide II).

nmr : δ (CDCl $_3$): 2.09 (4H, m, Pro C $^{\beta}$ H $_2$ + Pro C $^{\gamma}$ H $_2$), 3.50-4.03 (7H, s + m, COOCH $_3$ + Pro C $^{\delta}$ H $_2$ + Ser C $^{\beta}$ H $_2$), 4.66 (2H, m, Pro C $^{\alpha}$ H + Ser C $^{\alpha}$ H), 5.19 (2H, s, Z CH $_2$), 5.78 (1H, brd, Ser NH) 7.42 (5H, s, aromatic protons).

anal: Found: C, 58.34; H, 6.16; N, 8.34 %

Calc. for C $_{17}$ H $_{22}$ N $_2$ O $_6$: C, 58.28; H, 6.28; N, 8.00 %

$[\alpha]_D^{25}$: -79.15 (c, 1.66, MeOH).

(36) N-Benzylloxycarbonyl L-Seryl-L-Aspartic Acid Dimethyl Ester

Z-Ser-Asp(β -OMe)-OMe, 64):

Z-Ser-OH + H-Asp(β -OMe)-OMe.HCl $\xrightarrow{\text{Method VI}}$ Z-Ser-Asp(β -OMe)-OMe (64)

(i) Aspartic acid dimethyl ester hydrochloride (Asp(β -OMe)-OMe.HCl):

(82%)

mp. : 116-117 $^{\circ}$ C (lit. 144 mp. 116-117 $^{\circ}$ C)

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3450 (br), 1750 (ester).

nmr : δ (CDCl_3): 3.3 (2H, d, Asp C^βH_2), 3.72, 3.82 (3H, 3H, s, $\text{COOCH}_3 \times 2$), 4.70 (1H, m, Asp C^αH), 8.72 (2H, br, NH_2).

(ii) Z-Ser-Asp(β -OMe)-OMe (64): (58%)

mp. : 97-98°C

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3314 (NH), 1727 (ester), 1685 (carbamate), 1649 (amide I), 1549 (amide II), 1526 (amide II).

nmr : δ (60 MHz, CDCl_3): 2.90 (2H, d, $J=5.5$ Hz, Asp C^βH_2), 3.63, 3.73 (3H, 3H, s, s, $\text{COOCH}_3 \times 2$), 3.90 (2H, m, Ser C^βH_2), 4.16 (1H, m, Ser C^αH), 4.76 (1H, m, Asp C^αH), 5.10 (2H, s, Z CH_2), 5.83 (1H, br, Ser NH), 7.26 (6H, s + m, Asp NH + aromatic protons).

anal: Found: C, 53.78; H, 5.57; N, 7.23 %

Calc. for $\text{C}_{17}\text{H}_{22}\text{N}_2\text{O}_8$: C, 53.40; H, 5.76; N, 7.33 %

$[\alpha]_D^{25}$: -14.77 (c, 3.33, MeOH).

(37) N-Benzyloxycarbonyl L-Seryl-L-Serine Methyl Ester (Z-Ser-Ser-OMe,

66):

Z-Ser-OH + H-Ser-OMe.HCl $\xrightarrow{\text{Method VI}}$ Z-Ser-Ser-OMe (66)

Z-Ser-Ser-OMe (66): (60%)

mp. : 136-139°C (lit.¹⁴⁵ mp. 143-145°C)

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3445 (OH), 3305 (NH), 3280 (NH), 1738 (ester), 1663 (amide I), 1636 (amide I), 1547 (amide II).

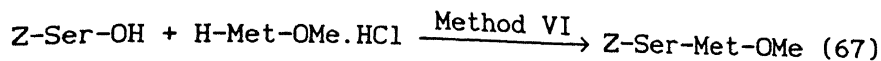
nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 3.68-3.93 (7H, s+br, Ser $\text{C}^\beta\text{H}_2 \times 2$ + COOCH_3), 4.44 (2H, m, Ser $\text{C}^\alpha\text{H} \times 2$), 5.04 (2H, s, Z CH_2), 6.44 (1H, d, $J=7.5$ Hz, Ser NH(Z)), 7.25 (5H, s, aromatic protons), 7.61 (1H, d, $J=7.5$ Hz, Ser NH).

anal: Found: C, 53.08; H, 5.96; N, 8.41 %

Calc. for $\text{C}_{15}\text{H}_{20}\text{N}_2\text{O}_7$: C, 52.94; H, 5.88; N, 8.23 %

$[\alpha]_D^{25}$: -4.2 (c, 3.3, MeOH).

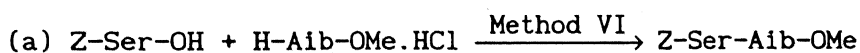
(38) N-Benzyloxycarbonyl L-Seryl-L-Methionine Methyl Ester (Z-Ser-Met-OMe,

67):

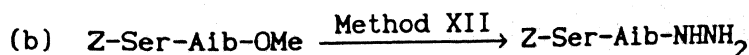
(i) Methionine methyl ester hydrochloride (Met-OMe.HCl): (82%)

mp. : 150°C (lit.¹⁴⁶ mp. 151°C)ir : ν_{max} (KBr) cm^{-1} : 1730 (ester).(ii) Z-Ser-Met-OMe (67): (63%)mp. : $98-99^{\circ}\text{C}$ (lit.¹⁴⁷ mp. $101-102^{\circ}\text{C}$)ir : ν_{max} (KBr) cm^{-1} : 3304 (NH), 1756 (ester), 1695 (carbamate),
1656 (amide I), 1545 (amide II).nmr : δ (60 MHz, CDCl_3): 2.06 (5H, s+m, Met $\text{C}^{\beta}\text{H}_2$ + Met S- CH_3),
2.46 (2H, m, Met $\text{C}^{\gamma}\text{H}_2$), 3.73 (3H, s, COOCH_3), 3.86 (2H, m,
Ser $\text{C}^{\beta}\text{H}_2$), 4.20 (1H, m, Ser $\text{C}^{\alpha}\text{H}$), 4.63 (1H, m, Met $\text{C}^{\alpha}\text{H}$),
5.01 (2H, s, Z CH_2), 5.86 (1H, brd, Ser NH), 7.43 (6H, s+m,
Met NH + aromatic protons).

anal: Found: C, 53.52; H, 6.27; N, 7.63 %

Calc. for $\text{C}_{17}\text{H}_{24}\text{N}_2\text{O}_6\text{S}$: C, 53.12; H, 6.25; N, 7.29 % $[\alpha]_{\text{D}}^{25}$: -25.42 (c, 1.66, MeOH).(39) N-Benzoyloxycarbonyl L-Seryl- α -Aminoisobutyryl-L-Serine Methyl Ester(Z-Ser-Aib-Ser-OMe, 69):(i) α -Aminoisobutyric acid methyl ester hydrochloride (Aib-OMe.HCl):
(96%)mp. : $180-181^{\circ}\text{C}$ (ii) N-Benzoyloxycarbonyl L-seryl- α -aminoisobutyric acid methyl ester
(Z-Ser-Aib-OMe): (92%)

mp. : gummy solid

Z-Ser-Aib-NHNH₂: (94%)mp. : $128-130^{\circ}\text{C}$ ir : ν_{max} (KBr) cm^{-1} : 3270 (br, NH), 1680 (br, amide I), 1640

(br, amide I), 1610, 1530 (br, amide II).

(c) Z-Ser-Aib-NHNH₂ + H-Ser-OMe.HCl

Method VII → Z-Ser-Aib-Ser-OMe (69)

Z-Ser-Aib-Ser-OMe (69): (47%)

mp. : 166-168°C

ir : ν_{\max} (KBr)cm⁻¹: 3441 (OH), 3306 (NH), 3275 (NH), 1749 (ester), 1671 (carbamate), 1648 (amide I), 1627 (amide I), 1560 (amide II).

nmr : δ (CDCl₃): 1.53 (6H, s, s, Aib CH₃x2), 3.84 (3H, s, COOCH₃), 4.03 (4H, m, Ser C ^{β} H₂x2), 4.20 (1H, m, Ser C ^{α} H), 4.64 (1H, m, Ser C ^{α} H), 5.22 (2H, s, Z CH₂), 6.03 (1H, d, J=7.5 Hz, Ser NH(Z)), 7.00 (1H, s, Aib NH), 7.15 (1H, m, Ser NH), 7.46 (5H, s, aromatic protons).

anal: Found: C, 53.27; H, 6.53; N, 9.64 %

Calc. for C₁₉H₂₇N₃O₈: C, 53.65; H, 6.35; N, 9.88 %

(40) N-Benzylloxycarbonyl L-Seryl-L-Leucyl-L-Serine Methyl Ester

(Z-Ser-Leu-Ser-OMe, 71):

(i) Z-Ser-Leu-OMe (55) Method XII → Z-Ser-Leu-NHNH₂

Z-Ser-Leu-NHNH₂ : (99%)

mp. : 178-179°C

(ii) Z-Ser-Leu-NHNH₂ + H-Ser-OMe.HCl Method VII → Z-Ser-Leu-Ser-OMe (71)

Z-Ser-Leu-Ser-OMe (71): (60%)

mp. : 182-183°C

ir : ν_{\max} (KBr)cm⁻¹: 3440 (OH), 3280 (NH), 1735 (ester), 1683 (carbamate), 1638 (amide I), 1615, 1535 (amide II).

nmr : δ [CDCl₃ + (CD₃)₂SO]: 0.87 (6H, d, J=5.0 Hz, Leu CH₃x2), 1.62 (3H, m, Leu C ^{β} H₂ + Leu C ^{γ} H), 3.71 (7H, s + m, COOCH₃ + Ser C ^{β} H₂x2), 4.12-4.89 (3H, m, Leu C ^{α} H + Ser C ^{α} Hx2), 5.09 (2H, s, Z CH₂), 6.71 (1H, d, J=7.5 Hz, Ser NH(Z)), 7.39 (5H, s, aromatic protons), 7.85 (2H, m, Leu NH + Ser NH).

ms : m/z: 454 (MH)⁺.

anal: Found: C, 55.37; H, 6.48; N, 9.38 %

Calc. for C₂₁H₃₁N₃O₈: C, 55.63; H, 6.84; N, 9.27 %

[α]_D²⁵: -39.25 (c, 0.21, MeOH).

(41) N-Benzyloxycarbonyl L-Seryl-Glycyl-L-Serine Methyl Ester

(Z-Ser-Gly-Ser-OMe, 73):

(i) Z-Ser-Gly-OMe (49) $\xrightarrow{\text{Method XII}}$ Z-Ser-Gly-NHNH₂

Z-Ser-Gly-NHNH₂ : (85%)

mp. : 173-174°C

ir : ν_{max} (KBr)cm⁻¹: 3295 (NH), 1684 (carbamate), 1650 (amide I), 1605, 1539 (amide II).

(ii) Z-Ser-Gly-NHNH₂ + H-Ser-OMe.HCl $\xrightarrow{\text{Method VII}}$ Z-Ser-Gly-Ser-OMe (73)

Z-Ser-Gly-Ser-OMe (73): (60%)

mp. : 171-172°C (crystallized from MeOH; lit.¹⁴⁸ mp. 173°C)

ir : ν_{max} (KBr)cm⁻¹: 3470 (OH), 3390 (NH), 3315 (NH), 1732 (ester), 1680 (carbamate), 1650 (amide I), 1548 (amide II), 1512.

nmr : δ [CDCl₃ + (CD₃)₂SO]: 3.56-4.03 (9H, s + m, COOCH₃ + Ser C^βH₂×2 + Gly CH₂), 4.37-4.87 (2H, m, Ser C^αH×2), 5.06 (2H, s, Z CH₂), 6.78 (1H, br, exchangeable with D₂O, Ser NH(Z)), 7.31 (5H, s, aromatic protons), 7.75 (1H, d, J=7.5 Hz, exchangeable, Ser NH), 8.09 (1H, t, exchangeable, Gly NH).

anal: Found: C, 51.37; H, 5.43; N, 10.36 %

Calc. for C₁₇H₂₃N₃O₈: C, 51.38; H, 5.79; N, 10.58 %

[α]_D²⁵: -11.20 (c, 0.5, MeOH).

(42) N-Benzyloxycarbonyl L-Seryl-L-Prolyl-L-Serine Methyl Ester

(Z-Ser-Pro-Ser-OMe, 75):

(i) Z-Ser-Pro-OMe (62) $\xrightarrow{\text{Method XII}}$ Z-Ser-Pro-NHNH₂

Z-Ser-Pro-NHNH₂ : (76%)

mp. : 130-131°C

ir : ν_{\max} (KBr) cm^{-1} : 3322, 3210, 3038, 1690 (carbamate), 1624 (amide I), 1558 (amide II).

(ii) Z-Ser-Pro-NHNH₂ + H-Ser-OMe.HCl $\xrightarrow{\text{Method VII}}$ Z-Ser-Pro-Ser-OMe (75)

Z-Ser-Pro-Ser-OMe (75): (30%)

mp. : syrup

ir : ν_{\max} (KBr) cm^{-1} : 3381 (NH), 1742 (ester), 1719 (carbamate), 1639 (amide I), 1533 (amide II), 1452.

nmr : δ (CDCl₃): 2.00 (4H, br, Pro C ^{β} H₂ + Pro C ^{γ} H₂), 3.07-4.00 (9H, s + m, COOCH₃ + Pro C ^{δ} H₂ + Ser C ^{β} H₂x2), 4.53 (3H, m, Pro C ^{α} H + Ser C ^{α} Hx2), 5.03 (2H, s, Z CH₂), 6.29 (1H, d, J=7.5 Hz, exchangeable with D₂O, Ser NH(Z)), 7.28 (5H, s, aromatic protons), 7.68 (1H, d, J=7.5 Hz, exchangeable, Ser NH).

anal: Found: C, 55.19; H, 6.33; N, 9.45 %

Calc. for C₂₀H₂₇N₃O₈: C, 54.92; H, 6.18; N, 9.61 %

$[\alpha]_{\text{D}}^{25}$: -65.53 (c, 3.16, MeOH).

(43) N-Benzyloxycarbonyl L-Threonyl-Glycine Methyl Ester (Z-Thr-Gly-OMe, 78):

Z-Thr-OH + H-Gly-OMe.HCl $\xrightarrow{\text{Method VI}}$ Z-Thr-Gly-OMe (78)

(i) N-Benzyloxycarbonyl threonine (Z-Thr-OH): (89%)

mp. : 99-100°C (lit.¹⁴⁹ mp. 101-102°C)

(ii) Z-Thr-Gly-OMe (78): (78%)

mp. : 94-97°C (lit.¹⁵⁰ mp. 105-106°C)

ir : ν_{\max} (KBr) cm^{-1} : 3288 (NH), 1731 (ester), 1689 (carbamate), 1649 (amide I), 1556 (amide II).

nmr : δ (CDCl₃): 1.15 (3H, d, J=6.5 Hz, Thr CH₃), 3.71 (3H, s, COOCH₃), 3.92 (2H, d, J=5.0 Hz, Gly CH₂), 4.21 (2H, m, Thr C ^{α} H + Thr C ^{β} H), 5.09 (2H, s, Z CH₂), 6.06 (1H, d, J=7.5 Hz, Thr NH), 7.34 (6H, s + m, Gly NH + aromatic protons).

anal: Found: C, 55.09; H, 6.42; N, 8.36 %

Calc. for $C_{15}H_{20}N_2O_6$: C, 55.55; H, 6.17; N, 8.64 %
 $[\alpha]_D^{25}$: -12.3 (c, 2.2, MeOH).

(44) N-Benzylloxycarbonyl L-Threonyl-L-Alanine Methyl Ester (Z-Thr-Ala-OMe, 79):

Z-Thr-OH + H-Ala-OMe.HCl $\xrightarrow{\text{Method VI}}$ Z-Thr-Ala-OMe (79)

Z-Thr-Ala-OMe (79): (60%)

mp. : 127-128°C (lit.¹⁵¹ mp. 132-134°C)

ir : ν_{\max} (KBr) cm^{-1} : 3404 (OH), 3300 (NH), 3065, 1756 (ester),
 1692 (carbamate), 1648 (amide I), 1542 (amide II).

nmr : δ (CDCl_3): 1.14 (3H, d, $J=6.5$ Hz, Thr CH_3), 1.34 (3H, d, $J=7.0$ Hz, Ala CH_3), 3.75 (3H, s, COOCH_3), 4.22 (2H, m, Thr C^αH + Thr C^βH), 4.53 (1H, m, Ala C^αH), 5.12 (2H, s, Z CH_2), 6.00 (1H, d, $J=7.5$ Hz, Thr NH), 7.35 (6H, s + m, Ala NH + aromatic protons).

anal: Found: C, 57.09; H, 6.43; N, 8.38 %

Calc. for $C_{16}H_{22}N_2O_6$: C, 56.80; H, 6.51; N, 8.28 %
 $[\alpha]_D^{25}$: -28.3 (c, 3.3, MeOH).

(45) N-Benzylloxycarbonyl L-Threonyl-L-Phenylalanine Methyl Ester
 (Z-Thr-Phe-OMe, 80):

Z-Thr-OH + H-Phe-OMe.HCl $\xrightarrow{\text{Method VI}}$ Z-Thr-Phe-OMe (80)

Z-Thr-Phe-OMe (80): (63%)

mp. : 98-99°C (lit.¹⁵² mp. 105-106°C)

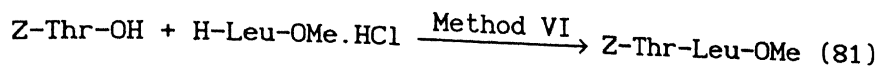
ir : ν_{\max} (KBr) cm^{-1} : 3299 (NH), 3063, 1743 (ester), 1697
 (carbamate), 1648 (amide I), 1540 (amide II).

nmr : δ (CDCl_3): 1.09 (3H, d, $J=6.5$ Hz, Thr CH_3), 3.06 (2H, m, Phe C^βH_2), 3.68 (3H, s, COOCH_3), 4.15 (2H, m, Thr C^αH + Thr C^βH), 4.81 (1H, m, Phe C^αH), 5.06 (2H, s, Z CH_2), 5.62 (1H, d, $J=7.5$ Hz, Thr NH), 6.75-7.56 (11H, s + m, Phe NH + aromatic protons).

anal: Found: C, 63.38; H, 6.59; N, 6.95 %

Calc. for $C_{22}H_{26}N_2O_6$: C, 63.77; H, 6.28; N, 6.76 %
 $[\alpha]_D^{25}$: -2.8 (c, 3.3, MeOH).

(46) N-Benzylloxycarbonyl L-Threonyl-L-Leucine Methyl Ester (Z-Thr-Leu-OMe, 81):



Z-Thr-Leu-OMe (81): (75%)

mp. : syrup (lit.¹⁵³ mp. 48-53°C)

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3415 (OH), 3322 (NH), 1739 (ester), 1692 (carbamate), 1650 (amide I), 1542 (amide II).

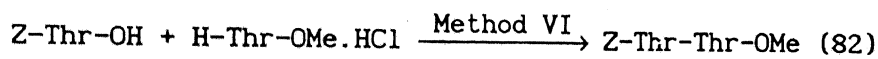
nmr : δ (CDCl_3): 0.90 (6H, d, $J=5.0$ Hz, Leu $\text{CH}_3 \times 2$), 1.15 (3H, d, $J=6.5$ Hz, Thr CH_3), 1.59 (3H, m, Leu C^βH_2 + Leu C^γH), 3.72 (3H, s, COOCH_3), 4.22 (2H, m, Thr C^αH + Thr C^βH), 4.50 (1H, m, Leu C^αH), 5.10 (2H, s, Z CH_2), 5.88 (1H, d, $J=7.5$ Hz, Thr NH), 7.00 (1H, brd, $J=7.5$ Hz, Leu NH), 7.31 (5H, s, aromatic protons).

anal: Found: C, 60.19; H, 7.46; N, 7.24 %

Calc. for $C_{19}H_{28}N_2O_6$: C, 60.00; H, 7.37; N, 7.37 %

(47) N-Benzylloxycarbonyl L-Threonyl-L-Threonine Methyl Ester

(Z-Thr-Thr-OMe, 82):



Z-Thr-Thr-OMe (82): (59%)

mp. : 98-99°C (lit.¹⁵⁴ mp. 67-68°C)

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3472 (OH), 3408 (OH), 3312 (NH), 1738 (ester), 1703 (carbamate), 1664 (amide I), 1549 (amide II).

nmr : δ (CDCl_3): 1.18 (6H, d, $J=6.5$ Hz, Thr $\text{CH}_3 \times 2$), 3.74 (3H, s, COOCH_3), 4.28 (2H, m, Thr $\text{C}^\beta\text{H} \times 2$), 4.53 (2H, dd, $J=8.7$ Hz, 2.5 Hz, Thr $\text{C}^\alpha\text{H} \times 2$), 5.12 (2H, s, Z CH_2), 6.09 (1H, d, $J=7.5$ Hz, Thr NH(Z)), 7.37 (6H, s+m, Thr NH + aromatic protons).

anal: Found: C, 55.64; H, 6.46; N, 7.46 %

Calc. for $C_{17}H_{24}N_2O_7$: C, 55.43; H, 6.52; N, 7.61 %

$[\alpha]_D^{25}$: -10.7 (c, 3.6, MeOH).

(48) N-Benzylloxycarbonyl L-Threonyl-(N^ω-Benzylloxycarbonyl)L-Lysine Methyl Ester (Z-Thr-Lys(N^ωZ)-OMe, 83):

Z-Thr-OH + H-Lys(N^ωZ)-OMe.HCl $\xrightarrow{\text{Method VI}}$ Z-Thr-Lys(N^ωZ)-OMe (83)

(i) N^ω-Benzylloxycarbonyl lysine methyl ester hydrochloride

(a) N^ω-Benzylloxycarbonyl lysine (N^ωZ-Lys):

A mixture of lysine monohydrochloride (9.1g, 50 mmol) and basic cupric carbonate (CuCO₃·Cu(OH)₂) (16.57g, 75 mmol) in water (350 mL) was refluxed for 2h, filtered hot, filtrate admixed with solid NaHCO₃ (16.8g, 200 mmol) and benzyloxycarbonyl chloride (95%) (7.11 mL, 50 mmol) and left stirred for 24h at room temperature, resulting blue solid filtered, dried in air, dissolved in minimum volume of 6N HCl, treated with EDTA solution (6.71g, 23 mmol) in 4N NaOH and the precipitated solid filtered and dried to give 11g (79%) of N^ωZ-Lys.

mp. : 250°C (lit.¹⁷⁰ mp. 250°C)

ir : ν_{max} (KBr)cm⁻¹: 3332 (NH), 1687 (carbamate), 1590, 1520.

(b) N^ωZ-Lys-OMe.HCl:

To an ice salt-cooled and stirred dry MeOH (15 mL) was added, in drops, freshly distilled SOCl₂ (0.8 mL, 10 mmol), followed by N^ωZ-Lys (2.8g, 10 mmol). The reaction mixture was stirred at room temperature for 2h, refluxed for 0.5h, solvents evaporated in vacuo to give N^ωZ-Lys-OMe.HCl (99%) as semisolid compound which was used as such for the next reaction.

mp. : sticky solid (lit.¹⁷⁰ mp. 117°C)

ir : ν_{max} (KBr)cm⁻¹: 3352, 1740 (ester), 1694 (carbamate), 1539.

nmr : δ (D₂O): 1.53 (6H, brm, Lys C^βH₂ + C^γH₂ + C^δH₂), 3.20 (2H, m, Lys C^ωH₂), 3.90 (3H, s, COOCH₃), 4.32 (1H, m, Lys C^αH), 5.10 (2H, s, ZCH₂), 7.40 (5H, s, aromatic protons).

(ii) Z-Thr-Lys(N^ωZ)-OMe (83): (59%)

mp. : 98-99°C (lit.¹⁵⁵)

ir : ν_{\max} (KBr) cm^{-1} : 3320 (NH), 1742 (ester), 1686 (carbamate), 1649 (amide I), 1541 (amide II).

nmr : δ (CDCl_3): 1.12 (3H, d, $J=6.5$ Hz, Thr CH_3), 1.40 (4H, m, Lys C^βH_2 + Lys $\text{C}^\gamma\text{H}_2$), 1.68 (2H, m, Lys $\text{C}^\delta\text{H}_2$), 3.12 (2H, m, Lys $\text{C}^\omega\text{H}_2$), 3.71 (3H, s, COOCH_3), 4.04-4.60 (3H, m, Thr C^αH + Thr C^βH + Lys C^αH), 5.04, 5.11 (2H, 2H, s, s, Z $\text{CH}_2 \times 2$), 5.78 (1H, d, $J=7.5$ Hz, Thr NH), 7.00 (1H, d, $J=7.5$ Hz, Lys N^ωH), 7.34 (11H, s + m, Lys NH + aromatic protons).

ms : m/z : 530 (MH) $^+$.

anal: Found: C, 61.44; H, 6.23; N, 7.88 %

Calc. for $\text{C}_{27}\text{H}_{35}\text{N}_3\text{O}_8$: C, 61.25; H, 6.62; N, 7.94 %

$[\alpha]_D^{25}$: -12.29 (c, 1.66, MeOH)

(49) N-^tButyloxycarbonyl L-Threonyl-L-Alanyl-L-Alanine Methyl Ester

(Boc-Thr-Ala-Ala-OMe, 85):

(i) Boc-Thr-OH + H-Ala-OMe.HCl $\xrightarrow{\text{Method VI}}$ Boc-Thr-Ala-OMe

N-^tButyloxycarbonyl L-threonyl L-alanine methyl ester

(Boc-Thr-Ala-OMe): (82%)

mp. : sticky solid

ir : ν_{\max} (KBr) cm^{-1} : 3384 (OH), 3309 (NH), 1745 (ester), 1660 (br, amide I), 1541 (amide II).

(ii) Boc-Thr-Ala-OMe $\xrightarrow{\text{Method IX}}$ Boc-Thr-Ala-OH

Boc-Thr-Ala-OH : (64%)

mp. : sticky solid

(iii) Boc-Thr-Ala-OH + H-Ala-OMe.HCl $\xrightarrow{\text{Method VI}}$ Boc-Thr-Ala-Ala-OMe (85)

Boc-Thr-Ala-Ala-OMe (85): (45%)

mp. : 127-128°C

ir : ν_{\max} (KBr) cm^{-1} : 3325 (NH), 1737 (ester), 1702 (carbamate), 1676 (amide I), 1635 (amide I), 1543 (amide II).

nmr : δ (CDCl_3): 1.15 (3H, d, $J=6.5$ Hz, Thr CH_3), 1.37 (6H, d, $J=7.5$ Hz, Ala $\text{CH}_3 \times 2$), 1.43 (9H, s, Boc $\text{CH}_3 \times 3$), 3.73 (3H, s,

COOCH_3), 4.00-4.72 (4H, m, Thr C^αH + Thr C^βH + Ala $\text{C}^\alpha\text{H}\times 2$),

5.53 (1H, d, $J=7.5$ Hz, Thr NH), 7.00 (2H, m, Ala $\text{NH}\times 2$).

anal: Found: C, 50.89; H, 7.67; N, 11.28 %

Calc. for $\text{C}_{16}\text{H}_{29}\text{N}_3\text{O}_7$: C, 51.20; H, 7.73; N, 11.20 %

(50) N-Benzylloxycarbonyl L-Threonyl-L-Alanyl-L-Alanine Methyl Ester

(Z-Thr-Ala-Ala-OMe, 87):

(i) Z-Thr-Ala-OMe (79) $\xrightarrow{\text{Method XII}}$ Z-Thr-Ala-NHNH₂

Z-Thr-Ala-NHNH₂ : (96%)

mp. : 195-196°C

ir : ν_{max} (KBr) cm^{-1} : 3300 (br, NH), 1685 (amide I), 1640 (br, amide I), 1595, 1530 (amide II).

(ii) Z-Thr-Ala-NHNH₂ + H-Ala-OMe.HCl $\xrightarrow{\text{Method VII}}$ Z-Thr-Ala-Ala-OMe (87)

Z-Thr-Ala-Ala-OMe (87): (42%)

mp. : 165-166°C

ir : ν_{max} (KBr) cm^{-1} : 3296 (NH), 1738 (ester), 1697 (carbamate), 1636 (amide I), 1551 (amide II),

nmr : δ (CDCl_3): 1.15 (3H, d, $J=6.5$ Hz, Thr CH_3), 1.34 (6H, d, $J=7.5$ Hz, Ala $\text{CH}_3\times 2$), 3.75 (3H, s, COOCH_3), 4.09-4.68 (4H, m, Thr C^αH + Thr C^βH + Ala $\text{C}^\alpha\text{H}\times 2$), 5.12 (2H, s, Z CH_2), 5.78 (1H, d, $J=7.5$ Hz, Thr NH), 7.34 (5H, s, aromatic protons), 7.84 (2H, m, Ala $\text{NH}\times 2$).

anal: Found: C, 55.92; H, 6.72; N, 10.38 %

Calc. for $\text{C}_{19}\text{H}_{27}\text{N}_3\text{O}_7$: C, 55.74; H, 6.60; N, 10.27 %

$[\alpha]_{\text{D}}^{25}$: -51.60 (c, 0.56, MeOH).

(51) N-Benzylloxycarbonyl L-Threonyl-(S-Benzyl)L-Cysteine Methyl Ester

(Z-Thr-Cys(S-Bzl)-OMe, 89):

Z-Thr-OH + H-Cys(S-Bzl)-OMe $\xrightarrow{\text{Method VI}}$ Z-Thr-Cys(S-Bzl)-OMe (89)

(i) S-Benzyl L-cysteine methyl ester hydrochloride (Cys(S-Bzl)-OMe.HCl):

A solution of 1.5g (7.1 mmol) of L-Cys(S-Bzl) in ~30 mL of 2N methanolic-HCl was left for 48 h at room temperature and then for 24 h

at 0-4°C. The clear solution was evaporated in vacuo, the residue obtained was crystallized from dry MeOH-Et₂O, the product washed several times with dry Et₂O and dried over P₂O₅ in desiccator.

mp. : 143°C (lit.¹⁵⁶ mp. 150°C)

ir : ν_{\max} (KBr)cm⁻¹: 1735 (ester)

(11) Z-Thr-Cys(S-Bzl)-OMe (89): (90%)

mp. : 140-141°C

ir : ν_{\max} (KBr)cm⁻¹: 3306 (NH), 1744 (ester), 1694 (carbamate), 1644 (amide I), 1530 (amide II).

nmr : δ (CDCl₃): 1.12 (3H, d, J=6.5 Hz, Thr CH₃), 2.81 (2H, m, Cys C ^{β} H₂), 3.69, 3.72 (5H, s, s, COOCH₃ + Bzl CH₂S), 3.93-4.50 (2H, m, Thr C ^{α} H + Thr C ^{β} H), 4.71 (1H, m, Cys C ^{α} H), 5.12 (2H, s, Z CH₂), 5.68 (1H, d, J=7.5 Hz, Thr NH), 7.12-7.56 (11H, s, Cys NH, aromatic protons).

ms : m/z: 461 (MH)⁺.

anal: Found: C, 59.87; H, 5.83; N, 6.38 %

Calc. for C₂₃H₂₈N₂O₆S: C, 60.00; H, 6.09; N, 6.09 %

$[\alpha]_D^{25}$: -26.14 (c, 0.83, MeOH).

(52) N-Benzyloxycarbonyl L-Threonyl-L-Leucyl-L-Leucine Methyl Ester

(Z-Thr-Leu-Leu-OMe, 91):

(1) Z-Thr-Leu-OMe (81) $\xrightarrow{\text{Method XII}}$ Z-Thr-Leu-NHNH₂

Z-Thr-Leu-NHNH₂ : (89%)

mp. : sticky solid

ir : ν_{\max} (KBr)cm⁻¹: 3330 (NH), 1682 (carbamate), 1620 (br, amide I), 1530 (amide II).

(11) Z-Thr-Leu-NHNH₂ + H-Leu-OMe.HCl $\xrightarrow{\text{Method VII}}$ Z-Thr-Leu-Leu-OMe (91)

Z-Thr-Leu-Leu-OMe (91): (59%)

mp. : 136-137°C

ir : ν_{\max} (KBr)cm⁻¹: 3290 (NH), 1746 (ester), 1699 (carbamate), 1640 (amide I), 1542 (amide II).

nmr : δ (60 MHz, CDCl_3): 0.90 (12H, brs, Leu $\text{CH}_3 \times 4$), 1.13 (3H, d, $J=6.5$ Hz, Thr CH_3), 1.59 (6H, m, Leu $\text{C}^\beta\text{H}_2 \times 2$ + Leu $\text{C}^\gamma\text{H} \times 2$), 3.66 (3H, s, COOCH_3), 4.03-4.76 (4H, m, Thr C^αH + Thr C^βH + Leu $\text{C}^\alpha\text{H} \times 2$), 5.06 (2H, s, Z CH_2), 5.96 (1H, brd, Thr NH), 6.73-7.46 (7H, s+m, Leu $\text{NH} \times 2$ + aromatic protons).

ms : m/z : 494 (MH)⁺.

anal: Found: C, 61.38; H, 7.84; N, 8.09 %

Calc. for $\text{C}_{25}\text{H}_{39}\text{N}_3\text{O}_7$: C, 60.85; H, 7.91; N, 8.52 %

$[\alpha]_D^{25}$: -45.84 (c, 1.66, MeOH).

(53) N-Benzoyloxycarbonyl L-Threonyl-L-Leucyl-L-Leucyl-L-Leucine Methyl Ester (Z-Thr-Leu-Leu-Leu-OMe, 93):

(i) Boc-Leu-OH + H-Leu-OMe.HCl $\xrightarrow{\text{Method VI}}$ Boc-Leu-Leu-OMe

(a) N-^tButyloxycarbonyl leucine (Boc-Leu-OH): (95%)

mp. : 85-86°C

(b) N-^tButyloxycarbonyl L-leucyl-L-leucine methyl ester

(Boc-Leu-Leu-OMe): (93%)

mp. : 128-129°C

ir : ν_{max} (KBr) cm^{-1} : 3352 (NH), 3276 (NH), 1760 (ester), 1684 (carbamate), 1657 (amide I), 1627 (amide I), 1560 (amide II), 1520 (amide II).

(ii) Boc-Leu-Leu-OMe $\xrightarrow{\text{Method X}}$ H-Leu-Leu-OMe

(iii) Z-Thr-Leu-NHNH₂ (Expt. 52) + H-Leu-Leu-OMe

$\xrightarrow{\text{Method VII}}$ Z-Thr-Leu-Leu-Leu-OMe (93)

Z-Thr-Leu-Leu-Leu-OMe (93): (52%)

mp. : 197-198°C

ir : ν_{max} (KBr) cm^{-1} : 3281 (NH), 1750 (ester), 1699 (carbamate), 1637 (amide I), 1543 (amide II).

nmr : δ (CDCl_3): 0.89 (18H, brs, Leu $\text{CH}_3 \times 6$), 1.11 (3H, d, $J=6.5$ Hz, Thr CH_3), 1.66 (9H, m, Leu $\text{C}^\beta\text{H}_2 \times 3$ + Leu $\text{C}^\gamma\text{H} \times 3$), 3.69 (3H, s, COOCH_3), 3.93-4.84 (5H, m, Thr C^αH + Thr C^βH + Leu

$C^{\alpha}Hx3$), 5.09 (2H, s, Z CH_2), 6.03 (1H, d, $J=7.5$ Hz, Thr NH), 7.43 (8H, s+m, Leu $NHx3$ + aromatic protons).

ms : m/z : 607 (MH)⁺.

anal: Found: C, 61.44; H, 8.08; N, 9.43 %

Calc. for $C_{31}H_{50}N_4O_8$: C, 61.39; H, 8.25; N, 9.24 %

$[\alpha]_D^{25}$: -61.5 (c, 1.66, MeOH).

(54) N-Benzoyl L-Serine Amide (Bz-Ser-NH₂, 95):

Bz-Ser-OMe $\xrightarrow{\text{Method XIII}}$ Bz-Ser-NH₂ (95)

Bz-Ser-NH₂ (95): (75%)

mp. : 162-163°C

ir : ν_{\max} (KBr) cm^{-1} : 3378 (NH), 3292 (NH), 3188 (NH), 1634 (amide I), 1578, 1525 (amide II).

(55) N-Benzylloxycarbonyl L-Serine Amide (Z-Ser-NH₂, 97):

Z-Ser-OMe $\xrightarrow{\text{Method XIII}}$ Z-Ser-NH₂ (97)

Z-Ser-NH₂ (97): (82%)

mp. : 130-131°C (lit.¹⁵⁷ mp. 132°C)

ir : ν_{\max} (KBr) cm^{-1} : 3376 (OH), 3318 (NH), 3204 (NH), 1686, 1650 (amide I), 1532 (amide II), 1465.

nmr : δ [(CD₃)₂SO]: 3.65 (2H, m, Ser $C^{\beta}H_2$), 4.06 (1H, m, Ser $C^{\alpha}H$), 5.06 (2H, s, Z CH_2) 6.56-7.46 (8H, s + m, Ser NH + CONH₂ + aromatic protons).

anal: Found: C, 55.65; H, 5.96; N, 11.89 %

Calc. for $C_{11}H_{14}N_2O_4$: C, 55.46; H, 5.88; N, 11.76 %

$[\alpha]_D^{26}$: +7.95 (c, 1.66, MeOH).

(56) N-Benzylloxycarbonyl L-Leucyl-L-Serine Amide (Z-Leu-Ser-NH₂, 98):

Z-Leu-Ser-OMe $\xrightarrow{\text{Method XIII}}$ Z-Leu-Ser-NH₂ (98)

Z-Leu-Ser-NH₂ (98): (74%)

mp. : 148-149°C

ir : ν_{\max} (KBr) cm^{-1} : 3299 (NH), 1689, 1646 (amide I), 1540 (amide II).

nmr : δ [$\text{CDCl}_3 + (\text{CD}_3)_2\text{SO}$]: 0.87 (6H, d, $J=5.0$ Hz, Leu $\text{CH}_3 \times 2$), 1.56 (3H, m, Leu $\text{C}^\beta\text{H}_2 + \text{Leu C}^\gamma\text{H}$), 3.68 (2H, m, Ser C^βH_2), 4.15 (1H, m, Ser C^αH), 4.80 (1H, m, Leu C^αH), 5.06 (2H, s, Z CH_2), 6.78-7.37 (8H, s + m, Leu NH + CONH_2 + aromatic protons), 7.65 (1H, d, $J=7.5$ Hz, Ser NH).

anal: Found: C, 58.24; H, 7.34; N, 11.80 %

Calc. for $\text{C}_{17}\text{H}_{25}\text{N}_3\text{O}_5$: C, 58.12; H, 7.12; N, 11.97 %

(57) N-Benzoyl Glycyl-L-Serine Amide (Bz-Gly-Ser-NH₂, 100):

Bz-Gly-Ser-OMe (7) $\xrightarrow{\text{Method XIII}}$ Bz-Gly-Ser-NH₂ (100)

Bz-Gly-Ser-NH₂ (100): (65%)

mp. : 110-111°C

ir : ν_{max} (KBr) cm^{-1} : 3382 (NH), 3283 (NH), 1661 (amide I), 1552 (amide II).

nmr : δ [$\text{CDCl}_3 + (\text{CD}_3)_2\text{SO}$]: 3.78 (2H, m, Ser C^βH_2), 3.96 (2H, d, $J=5.0$ Hz, Gly CH_2), 4.39 (1H, m, Ser C^αH), 4.80 (1H, br, exchangeable with D_2O , Ser OH), 6.84 (1H, br, Gly NH), 7.09-8.60 (8H, m, Ser NH + CONH_2 + aromatic protons).

anal: Found: C, 54.52; H, 5.72; N, 15.89 %

Calc. for $\text{C}_{12}\text{H}_{15}\text{N}_3\text{O}_4$: C, 54.34; H, 5.66; N, 15.85 %

(58) N-Benzyloxycarbonyl L-Threonine Amide (Z-Thr-NH₂, 102):

Z-Thr-OMe $\xrightarrow{\text{Method XIII}}$ Z-Thr-NH₂ (102)

Z-Thr-NH₂ (102): (60%)

mp. : sticky solid

ir : ν_{max} (KBr) cm^{-1} : 3280 (br, NH), 1670 (amide I), 1638 (amide I), 1600, 1525 (br, amide II).

(59) N-Benzyloxycarbonyl L-Seryl-L-Leucine Amide (Z-Ser-Leu-NH₂, 103):

Z-Ser-Leu-OMe (55) $\xrightarrow{\text{Method XIII}}$ Z-Ser-Leu-NH₂ (103)

Z-Ser-Leu-NH₂ (103): (90%)

mp. : 110-115°C

ir : ν_{max} (KBr) cm^{-1} : 3444 (OH), 3303 (NH), 1644 (br, amide I),

7.06-7.96 (8H, m, Leu NHx2 + Ser NH + aromatic protons).

anal: Found: C, 61.16; H, 7.83; N, 9.48 %

Calc. for $C_{23}H_{35}N_3O_6$: C, 61.47; H, 7.79; N, 9.35 %

$[\alpha]_D^{26}$: -25.9 (c, 3.3, $CHCl_3$).

(62) N-Benzoyl L-Alanyl-L-Seryl-L-Alanine Methyl Ester

(Bz-Ala-Ser-Ala-OMe, 107):

(i) Bz-Ala-Ser-OMe (9) $\xrightarrow{\text{Method XII}}$ Bz-Ala-Ser-NHNH₂

Bz-Ala-Ser-NHNH₂ : (89%)

mp. : 208-209°C

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3280 (NH), 1640 (amide I), 1605, 1567 (amide II), 1542 (amide II), 1520 (amide II).

(ii) Bz-Ala-Ser-NHNH₂ + H-Ala-OMe.HCl $\xrightarrow{\text{Method VII}}$ Bz-Ala-Ser-Ala-OMe (107)

Bz-Ala-Ser-Ala-OMe (107): (62%)

mp. : 195-196°C

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3270 (NH), 3070, 2980, 2930, 1743 (ester), 1688, 1625 (amide I), 1533 (amide II).

nmr : δ [400 MHz, $CDCl_3$ + $(CD_3)_2SO$]: 1.28, 1.36 (3H, 3H, d, d, J=7.2 Hz, 7.2 Hz, Ala CH_3 x2), 3.40 (2H, m, Ser $C^{\beta}H_2$), 3.60 (3H, s, $COOCH_3$), 4.28 (2H, m, Ala $C^{\alpha}H$ x2), 4.48 (1H, m, Ser $C^{\alpha}H$), 7.50 (3H, m, aromatic protons), 7.90 (3H, m, NH + aromatic protons), 8.16 (1H, d, J=7.5 Hz, NH), 8.54 (1H, d, J=7.5 Hz, NH).

ms : m/z: 365 (M)⁺.

anal: Found: C, 55.54; H, 6.27; N, 11.59 %

Calc. for $C_{17}H_{23}N_3O_6$: C, 55.89; H, 6.30; N, 11.51 %

$[\alpha]_D^{26}$: -30.96 (c, 1.76, MeOH).

(63) N-Benzyloxycarbonyl L-Leucyl-L-Seryl-L-Histidine Methyl Ester

(Z-Leu-Ser-His-OMe, 109):

(i) Z-Leu-OH + H-Ser-OMe.HCl $\xrightarrow{\text{Method VI}}$ Z-Leu-Ser-OMe

(a) N-Benzyloxycarbonyl leucine (Z-Leu-OH) : (93%)

mp. : syrup (lit.¹⁵⁸ mp. oil)

(b) Z-Leu-Ser-OMe : (89%)

mp. : 114-115°C (lit.¹⁵⁹ mp. 116°C)

(ii) Z-Leu-Ser-OMe $\xrightarrow{\text{Method XII}}$ Z-Leu-Ser-NHNH₂

Z-Leu-Ser-NHNH₂ : (96%)

mp. : 148-149°C

(iii) Z-Leu-Ser-NHNH₂ + H-His-OMe.2HCl

$\xrightarrow{\text{Method VII}}$ Z-Leu-Ser-His-OMe (109)

(a) Histidine methyl ester dihydrochloride (His-OMe.2HCl):

A mixture of L-His.HCl (10g, 52.14 mmol), dry MeOH (150 mL) and conc. H₂SO₄ (2.6 mL), was refluxed for 1h, subjected to passage of dry HCl for 2h, cooled, evaporated and the residue crystallized from MeOH/Et₂O to yield 11.2g (82%) of His-OMe.2HCl.

mp. : 198-199° (lit.¹⁶⁰ mp. 200-201°C)

ir : ν_{max} (KBr)cm⁻¹: 3100, 1750 (ester).

(b) Z-Leu-Ser-His-OMe (109): (68%)

mp. : 126-127°C

ir : ν_{max} (KBr)cm⁻¹: 3298 (NH), 1732 (ester), 1688 (carbamate), 1640 (amide I), 1537 (amide II).

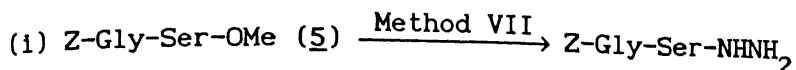
nmr : δ [CDCl₃ + (CD₃)₂SO]: 0.90 (6H, d, J=5.0 Hz, Leu CH₃x2), 1.62 (3H, m, Leu C ^{β} H₂ + Leu C ^{γ} H), 3.06 (2H, m, His C ^{β} H₂), 3.53-4.00 (5H, s + m, COOCH₃ + Ser C ^{β} H₂), 4.37 (1H, m, Ser C ^{α} H), 4.53-5.37 (4H, s + m, Z CH₂ + Leu C ^{α} H + His C ^{α} H), 6.50 (1H, d, J=7.5 Hz, Leu NH), 6.81 (1H, s, Imidazolyl ⁴H), 7.18-8.15 (8H, s + m, His NH + Ser NH + Imidazolyl ²H + aromatic protons).

ms : m/z: 504 (MH)⁺.

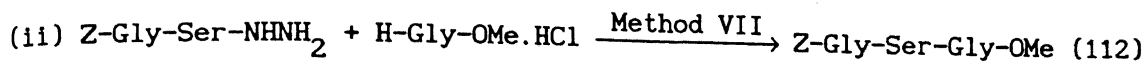
anal: Found: C, 57.43; H, 6.67; N, 13.49 %

Calc. for C₂₄H₃₃N₅O₇: C, 57.26; H, 6.56; N, 13.92 %

[α]_D²⁶: -15.66 (c, 1.66, MeOH).

(Z-Gly-Ser-Gly-OMe, 112):Z-Gly-Ser-NHNH₂: (88%)

mp. : 205°C

Z-Gly-Ser-Gly-OMe (112): (50%)

mp. : 154-155°C

ir : ν_{max} (KBr) cm⁻¹: 3297 (NH), 3066, 1760 (ester), 1704 (carbamate), 1646 (amide I), 1555 (amide II).

nmr : δ [CDCl₃ + (CD₃)₂SO]: 3.50-4.03 (9H, s + m, COOCH₃ + Ser C ^{β} H₂ + Gly CH₂x2), 4.53 (1H, m, Ser C ^{α} H), 5.12 (2H, s, Z CH₂), 7.00 (1H, br, Gly NH(Z)), 7.37 (5H, s, aromatic protons), 7.69 (1H, m, Gly NH), 8.00 (1H, m, Ser NH).

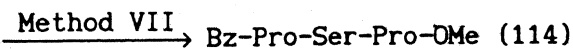
anal: Found: C, 52.49; H, 5.81; N, 11.38 %

Calc. for C₁₆H₂₁N₃O₇: C, 52.32; H, 5.72; N, 11.44 %[α]_D²⁶: -16.6 (c, 1.0, MeOH).

(65) N-Benzoyl L-Prolyl-L-Seryl-L-Proline Methyl Ester

(Bz-Pro-Ser-Pro-OMe, 114):Bz-Pro-Ser-NHNH₂: (90%)

mp. : sticky solid

Bz-Pro-Ser-Pro-OMe (114): (44%)

mp. : 70-71°C

ir : ν_{max} (KBr) cm⁻¹: 3340 (NH), 1735 (ester), 1625 (amide I), 1570 (amide II), 1530 (amide II), 1440.

nmr : δ (CDCl₃): 2.06 (8H, m, Pro C ^{β} H₂x2 + Pro C ^{γ} H₂x2), 3.40-4.03 (9H, s + m, COOCH₃ + Pro C ^{δ} H₂x2 + Ser C ^{β} H₂), 4.25-5.21 (3H, m, Ser C ^{α} H + Pro C ^{α} Hx2), 7.18-8.06 (6H, m,

Ser NH + aromatic protons).

anal: Found: C, 60.28; H, 6.35; N, 9.87 %

Calc. for $C_{21}H_{27}N_3O_6$: C, 60.43; H, 6.47; N, 10.07 %

$[\alpha]_D^{26}$: -42.52 (c, 1.74, $CHCl_3$).

(66) N-Benzoyl α -Aminoisobutyryl-L-Seryl- α -Aminoisobutyric Acid Methyl Ester (Bz-Aib-Ser-Aib-OMe, 116):

(i) Bz-Aib-OH + H-Ser-OMe.HCl $\xrightarrow{\text{Method VI}}$ Bz-Aib-Ser-OMe

(a) N-Benzoyl α -aminoisobutyric acid (Bz-Aib-OH):

mp. : 191-192°C

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3350 (NH), 1700, 1618, 1562, 1525.

(b) N-Benzoyl α -aminoisobutyryl-L-serine methyl ester

(Bz-Aib-Ser-OMe): (82%)

mp. : syrup

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3365 (br, NH), 1735 (ester), 1650 (br, amide I), 1515 (br, amide II).

(ii) Bz-Aib-Ser-OMe $\xrightarrow{\text{Method XII}}$ Bz-Aib-Ser-NHNH₂

Bz-Aib-Ser-NHNH₂ : (93%)

mp. : 57-58°C

(iii) Bz-Aib-Ser-NHNH₂ + H-Aib-OMe.HCl

$\xrightarrow{\text{Method VII}}$ Bz-Aib-Ser-Aib-OMe (116)

Bz-Aib-Ser-Aib-OMe (116): (75%)

mp. : 73-74°C

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3290 (NH), 1737 (ester), 1649 (amide I), 1537 (amide II).

nmr : δ ($CDCl_3$): 1.66 (12H, m, Aib $CH_3 \times 4$), 3.69 (3H, s, $COOCH_3$), 3.87-4.56 (3H, m, Ser $C^{\beta}H_2$ + Ser $C^{\alpha}H$), 7.10-8.00 (8H, m, Ser NH + Aib $NH \times 2$ + aromatic protons).

ms : m/z: 394 (MH)⁺.

anal: Found: C, 57.91; H, 6.56; N, 10.59 %

Calc. for $C_{19}H_{27}N_3O_6$: C, 58.01; H, 6.87; N, 10.69 %

$[\alpha]_D^{26}$: -6.89 (c, 2.13, EtOH).

(67) N-Benzylloxycarbonyl L-Leucyl-L-Seryl-L-Seryl-L-Leucyl-L-Leucyl-L-Seryl-L-Leucine Methyl Ester (Z-Leu-Ser-Ser-Leu-Leu-Ser-Leu-OMe, 119):

(i) Z-Leu-Ser-NHNH₂ (Expt. 63) + H-Ser-OMe.HCl

Method VII
→ Z-Leu-Ser-Ser-OMe

Z-Leu-Ser-Ser-OMe was directly used for further reaction.

(ii) Z-Leu-Ser-Ser-OMe $\xrightarrow{\text{Method XII}}$ Z-Leu-Ser-Ser-NHNH₂

Z-Leu-Ser-Ser-NHNH₂ : (76%)

mp. : 209-210°C

(iii) Z-Leu-Ser-Ser-NHNH₂ + H-Leu-Leu-OMe (Expt. 53)

Method VII
→ Z-Leu-Ser-Ser-Leu-Leu-OMe

Z-Leu-Ser-Ser-Leu-Leu-OMe was directly used for further reaction.

(iv) Z-Leu-Ser-Ser-Leu-Leu-OMe

Method XII
→ Z-Leu-Ser-Ser-Leu-Leu-NHNH₂ (118)

Z-Leu-Ser-Ser-Leu-Leu-NHNH₂ (118): (63%)

mp. : 172-173°C

ir : ν_{max} (KBr)cm⁻¹: 3293 (br, NH), 1653 (br, amide I), 1541 (amide II).

nmr : δ [400 MHz, (CD₃)₂SO]: 0.82 (18H, m, Leu CH₃x6), 1.34-1.68 (9H, m, Leu C ^{β} H₂x3 + Leu C ^{γ} Hx3), 3.44-3.70 (4H, m, Ser C ^{β} H₂x2), 4.02-4.38 (5H, m, C ^{α} Hx5), 5.02 (2H, s, Z CH₂), 5.16 (1H, br, Leu NH(Z)), 7.02 (2H, m, NHx2), 7.34 (5H, s, aromatic protons), 7.48 (1H, d, J=7.5 Hz, NH), 7.60 (1H, d, J=7.5 Hz, NH), 8.00 (3H, m, NHx3).

(v) Z-Ser-Leu-OMe $\xrightarrow{\text{Method XI}}$ H-Ser-Leu-OMe

(vi) Z-Leu-Ser-Ser-Leu-Leu-NHNH₂ (118) + H-Ser-Leu-OMe

Method VII
→ Z-Leu-Ser-Ser-Leu-Leu-Ser-Leu-OMe (118)

Z-Leu-Ser-Ser-Leu-Leu-Ser-Leu-OMe (119): (41%)

mp. : 145-146°C

ir : ν_{max} (KBr)cm⁻¹: 3291 (br, NH), 1748 (ester), 1694 (amide I), 1648 (amide I), 1534 (amide II).

nmr : δ [400 MHz, (CD₃)₂SO]: 0.93 (24H, brd, Leu CH₃x8), 1.66

(12H, m, Leu C^βH₂×4 + Leu C^γH×4), 3.73 (3H, brs, COOCH₃), 4.00 (2H, m, Ser C^βH₂), 4.20 (4H, m, Ser C^βH₂×2), 4.46 (4H, m, C^αH×4), 5.15 (5H, s+m, Z CH₂ + C^αH×3), 5.66 (1H, br, NH), 6.26 (1H, br, NH), 7.08-7.84 (9H, s+m, NH×4 + aromatic protons), 8.35 (1H, br, NH).

anal: Found: C, 57.44; H, 7.58; N, 11.36 %

Calc. for C₄₂H₆₉N₇O₁₃: C, 57.34; H, 7.85; N, 11.15 %

(68) N-Benzoyl L-Alanyl-L-Threonyl-L-Alanine Methyl Ester

(Bz-Ala-Thr-Ala-OMe, 120):

(i) Bz-Ala-Thr-OMe (42) $\xrightarrow{\text{Method IX}}$ Bz-Ala-Thr-OH

Bz-Ala-Thr-OH : (89%)

mp. : 154-155°C

ir : ν_{max} (KBr)cm⁻¹: 3330 (br), 1615 (br, amide I), 1535 (br, amide II).

(ii) Bz-Ala-Thr-OH + H-Ala-OMe.HCl $\xrightarrow{\text{Method VI}}$ Bz-Ala-Thr-Ala-OMe (120)

Bz-Ala-Thr-Ala-OMe (120): (65%)

mp. : 206-207°C

ir : ν_{max} (KBr)cm⁻¹: 3326 (NH), 3268 (NH), 1738 (ester), 1683 (amide I), 1623 (amide I), 1577 (amide II), 1532 (amide II).

nmr : δ (CDCl₃): 1.12 (3H, d, J=6.5 Hz, Thr CH₃), 1.40 (3H, d, J=7.5 Hz, Ala CH₃), 1.53 (3H, d, J=7.5 Hz, Ala CH₃), 3.78 (3H, s, COOCH₃), 4.15-4.96 (4H, m, Thr C^αH + Thr C^βH + Ala C^αH×2), 7.31-8.22 (8H, m, Thr NH + Ala NH×2 + aromatic protons).

anal: Found: C, 57.14; H, 6.26; N, 10.87 %

Calc. for C₁₈H₂₅N₃O₆: C, 56.99; H, 6.60; N, 11.08 %

[α]_D²⁶: -30.9 (c, 3.3, MeOH).

(69) N-Benzoyl L-Prolyl-L-Threonyl-L-Proline Methyl Ester

(Bz-Pro-Thr-Pro-OMe, 121):

(i) Bz-Pro-OH + H-Thr-OMe.HCl $\xrightarrow{\text{Method VI}}$ Bz-Pro-Thr-OMe

Bz-Pro-Thr-OMe : (86%)

mp. : 95-97°C

(ii) Bz-Pro-Thr-OMe $\xrightarrow{\text{Method IX}}$ Bz-Pro-Thr-OH

Bz-Pro-Thr-OH : (92%)

mp. : 128-129°C

ir : ν_{max} (KBr) cm^{-1} : 3325 (NH), 1615 (br, amide I), 1560 (amide II), 1525 (amide II).

(iii) Bz-Pro-Thr-OH + H-Pro-OMe.HCl $\xrightarrow{\text{Method VI}}$ Bz-Pro-Thr-Pro-OMe (121)

Bz-Pro-Thr-Pro-OMe (121): (83%)

mp. : syrup

ir : ν_{max} (KBr) cm^{-1} : 3326 (NH), 1745 (ester), 1626 (amide I), 1574 (amide II), 1533 (amide II), 1435.

nmr : δ (400 MHz, CDCl_3): 1.26 (3H, d, $J=6.5$ Hz, Thr CH_3), 1.76-2.38 (8H, m, Pro $\text{C}^\beta\text{H}_2 \times 2$ + Pro $\text{C}^\gamma\text{H}_2 \times 2$), 3.40-3.94 (7H, s+m, COOCH_3 + Pro $\text{C}^\delta\text{H}_2 \times 2$), 4.18 (1H, m, Thr C^βH), 4.52 (1H, m, Thr C^αH), 4.72 (2H, m, Pro $\text{C}^\alpha\text{H} \times 2$), 7.26-7.64 (6H, m, Thr NH + aromatic protons).

ms : m/z : 432 (MH) $^+$.

anal: Found: C, 61.67; H, 7.03; N, 9.93 %

Calc. for $\text{C}_{22}\text{H}_{29}\text{N}_3\text{O}_6$: C, 61.25; H, 6.73; N, 9.74 %

(70) N-Benzoyl L-Alanyl-L-Alanyl-L-Threonyl-L-Alanyl-L-Alanine Methyl

Ester (Bz-Ala-Ala-Thr-Ala-Ala-OMe, 122):

(i) Bz-Ala-OH + H-Ala-OMe.HCl $\xrightarrow{\text{Method VI}}$ Bz-Ala-Ala-OMe

Bz-Ala-Ala-OMe : (81%)

mp. : 130-131°C

ir : ν_{max} (KBr) cm^{-1} : 3315, 3290, 1745 (ester), 1655, 1625, 1570, 1518.

(ii) Bz-Ala-Ala-OMe $\xrightarrow{\text{Method IX}}$ Bz-Ala-Ala-OH

Bz-Ala-Ala-OH : (92%)

mp. : 88-89°C

ir : ν_{\max} (KBr) cm^{-1} : 3453 (br, OH), 3305 (NH), 1674 (br, amide I), 1641 (amide I), 1534 (br, amide II).

(iii) Bz-Ala-Ala-OH + H-Thr-OMe.HCl $\xrightarrow{\text{Method VI}}$ Bz-Ala-Ala-Thr-OMe

Bz-Ala-Ala-Thr-OMe : (79%)

mp. : sticky solid

(iv) Bz-Ala-Ala-Thr-OMe $\xrightarrow{\text{Method XII}}$ Bz-Ala-Ala-Thr-NHNH₂

Bz-Ala-Ala-Thr-NHNH₂ : (94%)

mp. : 196-197°C

(v) Z-Ala-OH + H-Ala-OMe.HCl $\xrightarrow{\text{Method VI}}$ Z-Ala-Ala-OMe

(1) Z-Ala-OH : (92%)

mp. : 95-96°C (lit.¹⁶¹ mp. 97-99°C)

(2) Z-Ala-Ala-OMe: (89%)

mp. : 104-105°C

ir : ν_{\max} (KBr) cm^{-1} : 3301 (NH), 1738 (ester), 1687 (carbamate), 1647 (amide I), 1626 (amide I), 1574 (amide II), 1538 (amide II).

(vi) Z-Ala-Ala-OMe $\xrightarrow{\text{Method XI}}$ H-Ala-Ala-OMe

(vii) Bz-Ala-Ala-Thr-NHNH₂ + H-Ala-Ala-OMe

$\xrightarrow{\text{Method VII}}$ Bz-Ala-Ala-Thr-Ala-Ala-OMe (122)

Bz-Ala-Ala-Thr-Ala-Ala-OMe (122): (43%)

mp. : 253-254°C

ir : ν_{\max} (KBr) cm^{-1} : 3271 (NH), 3071, 1753 (ester), 1690, 1627 (amide I), 1531 (amide II).

nmr : δ [CDCl₃ + (CD₃)₂SO]: 0.90-1.56 (15H, m, Thr CH₃ + Ala CH₃x4), 3.71 (3H, s, COOCH₃), 4.09-4.87 (6H, m, Thr C ^{α} H + Thr C ^{β} H + Ala C ^{α} Hx4), 7.28-8.47 (10H, m, Thr NH + Ala NHx4 + aromatic protons).

ms : m/z: 522 (MH)⁺.

anal: Found: C, 55.11; H, 6.49; N, 13.74 %

Calc. for C₂₄H₃₅N₅O₈: C, 55.28; H, 6.72; N, 13.44 %

$[\alpha]_{\text{D}}^{26}$: -43.13 (c, 1.66, MeOH).

Ru(VIII) Oxidation of Ser/Thr Peptides: General Procedure

A typical procedure for the Ru(VIII) mediated oxidation of Ser/Thr peptides is as follows:

A mixture of the Ser/Thr peptide (1 mmol), NaIO_4 (18 mmol), $\text{RuCl}_3 \cdot 3\text{H}_2\text{O}$ (2.2 mol%) and $\text{MeCN}/\text{CCl}_4/\text{pH } 3$ phosphate buffer (or distilled water when pH-6 was required) :: 4mL/4mL/8mL was mechanically shaken in a sealed flask at room temperature for 1.5 h, cooled, cautiously opened, filtered, the residue washed with CH_3CN (2x5 mL), the combined filtrates evaporated in vacuo without heating, the residue stirred with saturated aq. NaHCO_3 (15 mL), extracted with EtOAc (3x20 mL), dried (MgSO_4) and solvents evaporated in vacuo to yield the crude product amide in the case of C-terminal Ser/Thr peptides and uncleaved product in the case of N-terminal or non-terminal Ser/Thr peptides. The products were either crystallized from hot EtOAc, MeOH or purified on a column of silica gel (100-200 mesh) using Benzene/EtOAc as eluents. In cases where acidic product was expected, the bicarbonate extract was cooled, acidified with 2N H_2SO_4 (pH~3), saturated with solid NaCl and extracted with EtOAc (2x30 mL), dried (MgSO_4), evaporated and crystallized or directly esterified with diazomethane in ether.

The structures of the product C-terminal amides were confirmed by direct comparison of their spectra and mp. with the authentic samples (prepared from peptide methyl esters and dry NH_3 gas).

Cleavage reactions were carried out at pH 3 for 1.5 h unless otherwise stated.

(71) Reaction of Bz-Ser-OMe (1) with Ru(VIII) : Isolation of Benzamide

(Bz-NH₂, 2):

Bz-NH₂ (2): (84%)

mp. : 126°C (lit.¹⁶² mp. 128-129°C)

(72) Reaction of o-NO₂Bz-Ser-OMe (3) with Ru(VIII) : Isolation of o-NO₂Bz-NH₂ (4):

o-NO₂Bz-NH₂ (4): (83%)

mp. : 173-174°C (lit.¹⁶⁷ mp. 174-178°C)

ir : ν_{\max} (KBr)cm⁻¹: 3363 (NH), 3176 (NH), 1656 (amide I), 1624 (amide I), 1575, 1524 (amide II, NO₂), 1352 (NO₂).

nmr : δ [CDCl₃ + (CD₃)₂SO]: 6.36-7.33 (2H, br, CONH₂), 7.60-8.23 (4H, m, aromatic protons).

ms : m/z: 167 (MH)⁺, 166 (M)⁺.

(73) Reaction of Z-Gly-Ser-OMe (5) with Ru(VIII) for 1.5h at pH 3 :

Isolation of Z-Glycyl-NH[oxalo]-Methyl Ester (Z-Gly-NH-CO-CO₂Me, 6):

Z-Gly-NH-CO-CO₂Me (6): (90%)

mp. : 92-93°C

ir : ν_{\max} (KBr)cm⁻¹: 3370 (NH), 3260 (NH), 3190 (NH), 1742 (ester), 1678 (carbamate), 1525 (amide II), 1490.

nmr : δ [CDCl₃ + (CD₃)₂SO]: 3.88 (3H, s, COOCH₃), 4.09 (2H, d, J=6.5 Hz, GlyCH₂), 5.13 (2H, s, ZCH₂), 7.06 (1H, br, exchangeable with D₂O, GlyNH), 7.38 (5H, s, aromatic protons), 11.25 (1H, s, exchangeable, CONHCO).

ms : m/z: 295 (MH)⁺.

anal: Found: C, 53.21; H, 4.47; N, 9.39 %

Calc. for C₁₃H₁₄N₂O₆: C, 53.06; H, 4.76; N, 9.52 %

(74) Reaction of Z-Gly-Ser-OMe (5) with Ru(VIII) for 8h at pH 3 :

Isolation of Z-Gly-NH₂ (6a):

Z-Gly-NH₂ (6a): (92%)

mp. : 131-132°C (lit.¹⁶³ mp. 135-136°C)

ir : ν_{\max} (KBr)cm⁻¹: 3382 (NH), 3327 (NH), 3184 (NH), 1680 (carbamate), 1642 (amide I), 1525 (amide II).

nmr : δ [CDCl₃ + (CD₃)₂SO]: 3.75 (2H, d, J=6.5 Hz, Gly CH₂), 5.09 (2H, s, Z CH₂), 6.34-7.16 (3H, br, Gly NH + CONH₂),

7.38 (5H, s, aromatic protons).

* Oxidation of Z-Gly-Ser-OMe (5) at pH 6 for 1.5h, gave 95% of Z-Gly-NH-CO-CO₂Me (6).

(75) Reaction of Bz-Gly-Ser-OMe (7) with Ru(VIII) : Isolation of

Bz-Gly-NH₂ (8):

Bz-Gly-NH₂ (8): (54%)

mp. : 170-171°C

ir : ν_{\max} (KBr)cm⁻¹: 3270 (NH), 3150 (NH), 3075, 2940, 1695 (amide I), 1675 (amide I), 1633 (amide I), 1603, 1575 (amide II), 1550 (amide II).

nmr : δ [CDCl₃ + (CD₃)₂SO]: 3.91 (2H, d, J=6.5 Hz, Gly CH₂), 6.65-8.25 (7H, m, CONH₂ + aromatic protons), 8.59 (1H, t, Gly NH).

(76) Reaction of Bz-Ala-Ser-OMe (9) with Ru(VIII) : Isolation of N-Benzoyl L-Alanine Amide (Bz-Ala-NH₂, 10):

Bz-Ala-NH₂ (10): (49%)

mp. : 232-234°C

ir : ν_{\max} (KBr)cm⁻¹: 3300 (NH), 3165 (NH), 1690 (amide I), 1635 (amide I), 1603, 1577 (amide II), 1548 (amide II).

anal: Found: C, 62.93; H, 6.16; N, 14.82 %

Calc. for C₁₀H₁₂N₂O₂: C, 62.50; H, 6.25; N, 14.58 %

$[\alpha]_D^{30}$: +21.1 (c, 1.7, MeOH).

(77) Reaction of Bz-Leu-Ser-OMe (11) with Ru(VIII) : Isolation of

N-Benzoyl L-Leucine Amide (Bz-Leu-NH₂, 12):

Bz-Leu-NH₂ (12): (68%)

mp. : 169-170°C

ir : ν_{\max} (KBr)cm⁻¹: 3390 (NH), 3325 (NH), 3195 (NH), 1635, (amide I), 1612 (amide I), 1588, 1562 (amide II), 1532 (amide II).

nmr : δ [CDCl₃ + (CD₃)₂SO]: 0.91 (6H, d, J=5.0 Hz, Leu CH₃x2),

1.69 (3H, m, Leu C^βH₂ + Leu C^γH), 4.75 (1H, m, Leu C^αH),
 5.60-6.82 (2H, br, CONH₂), 6.96 (1H, d, J=7.5 Hz, Leu NH),
 7.34-8.00 (5H, m, aromatic protons).

anal: Found: C, 67.08; H, 8.11; N, 11.77 %

Calc. for C₁₃H₁₈N₂O₂: C, 66.67; H, 7.69; N, 11.97 %

[α]_D³⁰: +2.1 (c, 1.6, CHCl₃).

* Reaction of Bz-Leu-Ser-OMe (11) with Ru(VIII) at pH 6 for 1.5h also afforded Bz-Leu-NH₂ (12) in 30% yields.

(78) Reaction of Bz-Phe-Ser-OMe (13) with Ru(VIII) : Isolation of N-Benzoyl L-Phenylalanine Amide (Bz-Phe-NH₂, 14):

Bz-Phe-NH₂ (14): (79%)

mp. : 183-184°C

ir : ν_{max} (KBr)cm⁻¹: 3410, 3335 (NH), 3200 (NH), 1662 (amide I),
 1635 (amide I), 1608, 1583 (amide II), 1525 (amide II).

anal: Found: C, 71.40; H, 6.22; N, 10.54 %

Calc. for C₁₆H₁₆N₂O₂: C, 71.64; H, 5.97; N, 10.45 %

[α]_D³⁰: -27.8 (c, 2.8, MeOH).

(79) Reaction of Bz-Asp(β-OMe)-Ser-OMe (15) with Ru(VIII) : Isolation of Bz-Aspartyl(β-OMe)-NH[oxalo]-Methyl Ester, 16):

Bz-Asp(β-OMe)-NH-CO-CO₂Me (16): (92%)

mp. : 156-157°C

ir : ν_{max} (KBr)cm⁻¹: 3271 (NH), 1782 (CONHCO), 1734 (ester),
 1633 (amide I), 1578 (amide II), 1531 (amide II), 1500.

nmr : δ [CDCl₃ + (CD₃)₂SO]: 2.93 (2H, m, Asp C^βH₂), 3.68, 3.87
 (3H, 3H, s, s, COOCH₃x2), 5.03 (1H, m, Asp C^αH), 7.25-8.06
 (5H, m, aromatic protons), 8.65 (1H, d, J=7.5 Hz, Asp NH),
 11.40 (1H, s, CONHCO).

ms : m/z: 337 (MH)⁺.

anal: Found: C, 53.73; H, 4.48; N, 8.26 %

Calc. for C₁₅H₁₆N₂O₇: C, 53.57; H, 4.76; N, 8.33 %

$[\alpha]_D^{30}$: -26.87 (c, 0.64, MeOH).

- (80) Reaction of Boc-Asp(β -OBzl)-Ser-OMe (17) with Ru(VIII) : Isolation of N-^tButyloxycarbonyl (β -OBzl)L-Aspartic Acid Amide (Boc-Asp(β -OBzl)-NH₂, 18):

Boc-Asp(β -OBzl)-NH₂ (18): (96%)

mp. : 145-146°C

ir : ν_{\max} (KBr)cm⁻¹: 3405 (NH), 3350 (NH), 3210 (NH), 1725 (Bzl ester), 1665 (amide I), 1635 (amide I), 1510 (amide II).

nmr : δ (CDCl₃): 1.43 (9H, s, Boc CH₃x3), 2.87 (2H, m, Asp C ^{β} H₂), 4.53 (1H, m, Asp C ^{α} H), 5.12 (2H, s, Bzl CH₂), 5.65 (1H, br, Asp NH), 6.40 (2H, br, CONH₂), 7.37 (5H, s, aromatic protons).

ms : m/z: 323 (MH)⁺.

anal: Found: C, 59.36; H, 6.63; N, 8.53 %

Calc. for C₁₆H₂₂N₂O₅: C, 59.63; H, 6.83; N, 8.70 %

$[\alpha]_D^{30}$: +33.83 (c, 0.13, CHCl₃).

- (81) Reaction of Bz-Glu(γ -OMe)-Ser-OMe (19) with Ru(VIII) : Isolation of N ^{α} Benzoyl Isoglutamine Methyl Ester (Bz-Glu(γ -OMe)-NH₂, 20):

Bz-Glu(γ -OMe)-NH₂ (20): (90%)

mp. : 136-137°C

ir : ν_{\max} (KBr)cm⁻¹: 3396 (NH), 3306 (NH), 3185 (NH), 1730 (ester), 1659 (amide I), 1633 (amide I), 1577 (amide II), 1523 (amide II).

nmr : δ [CDCl₃ + (CD₃)₂SO]: 1.87-2.56 (4H, m, Glu C ^{β} H₂ + Glu C ^{γ} H₂), 3.69 (3H, s, COOCH₃), 4.59 (1H, m, Glu C ^{α} H), 7.06-8.03 (8H, m, Glu NH + CONH₂ + aromatic protons).

ms : m/z: 264 (M)⁺.

anal: Found: C, 58.69; H, 5.76; N, 10.64 %

Calc. for C₁₃H₁₆N₂O₄: C, 59.09; H, 6.06; N, 10.61 %

- (82) Reaction of Z-Asn-Ser-OMe (21) with Ru(VIII) : Isolation of N^α-Benzyloxycarbonyl L-Asparagine Amide (Z-Asn-NH₂, 22):

Z-Asn-NH₂ (22): (65%)

mp. : 220-222°C (lit.¹⁶⁴ mp. 225-226°C)

ir : ν_{\max} (KBr) cm⁻¹: 3383 (NH), 3321 (NH), 3185 (NH), 1696 (amide I), 1655 (amide I), 1533 (amide II).

nmr : δ [CDCl₃ + (CD₃)₂SO]: 2.50 (2H, d, J=6.5 Hz, Asn C^βH₂), 4.34 (1H, m, Asn C^αH), 5.07 (2H, s, Z CH₂), 6.73-7.31 (10H, s+m, Asn NH + CONH₂×2 + aromatic protons).

ms : m/z: 266 (MH)⁺.

anal: Found: C, 54.25; H, 5.36; N, 15.75 %

Calc. for C₁₂H₁₅N₃O₄: C, 54.34; H, 5.66; N, 15.85 %

[α]_D³⁰: -2.32 (c, 0.56, MeOH).

- (83) Reaction of Z-Gln-Ser-OMe (23) with Ru(VIII) : Isolation of N^α-Benzyloxycarbonyl L-Glutamine Amide (Z-Gln-NH₂, 24):

Z-Gln-NH₂ (24): (90%)

mp. : 138-139°C

ir : ν_{\max} (KBr) cm⁻¹: 3390 (NH), 3315 (NH), 3199 (NH), 1654 (br, amide I), 1539 (amide II).

nmr : δ [CDCl₃ + (CD₃)₂SO]: 1.50-2.31 (4H, m, Gln C^βH₂ + Gln C^γH₂), 3.96 (1H, m, Gln C^αH), 5.09 (2H, s, Z CH₂), 6.64-7.50 (10H, s+m, Gln NH + CONH₂×2 + aromatic protons).

ms : m/z: 280 (MH)⁺.

anal: Found: C, 56.22; H, 6.04; N, 14.83 %

Calc. for C₁₃H₁₇N₃O₄: C, 55.91; H, 6.09; N, 15.05 %

[α]_D³⁰: +3.20 (c, 0.25, MeOH).

- (84) Reaction of Z-Met-Ser-OMe (25) with Ru(VIII) : Isolation of N-Benzyloxycarbonyl Methionine Sulfone Amide (Z-Met(SO₂)-NH₂, 26):

Z-Met(SO)₂-NH₂ (26): (95%)

mp. : 111-112°C

ir : ν_{\max} (KBr) cm^{-1} : 3425 (NH), 3380 (NH), 3190 (NH), 1650 (amide I), 1525 (amide II).

nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 2.00-3.34 (7H, s + m, COOCH_3 + Met C^βH_2 + Met $\text{C}^\gamma\text{H}_2$), 4.37 (1H, m, Met C^αH), 5.12 (2H, s, Z CH_2), 6.62 (1H, brd, Met NH), 7.40 (7H, s + m, CONH_2 + aromatic protons)

ms : m/z: 315 (MH) $^+$.

anal: Found: C, 49.43; H, 5.94; N, 8.63 %

Calc. for $\text{C}_{13}\text{H}_{18}\text{N}_2\text{O}_5\text{S}$: C, 49.68; H, 5.73; N, 8.92 %

$[\alpha]_{\text{D}}^{30}$: +5.65 (c, 0.56, MeOH).

(85) Reaction of Bz-Val-Ser-OMe (27) with Ru(VIII) : Isolation of N-Benzoyl L-Valine Amide (Bz-Val-NH $_2$, 28):

Bz-Val-NH $_2$ (28): (95%)

mp. : 216-217 $^{\circ}\text{C}$

ir : ν_{\max} (KBr) cm^{-1} : 3400 (NH), 3320 (NH), 3210 (NH), 1660 (amide I), 1632 (amide I), 1602, 1578 (amide II), 1520 (amide II).

nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 1.00 (6H, dd, J=5.0 Hz, 2.5 Hz, Val $\text{CH}_3 \times 2$), 2.15 (1H, m, Val C^βH), 4.50 (1H, m, Val C^αH), 7.12-7.96 (8H, m, Val NH + CONH_2 + aromatic protons).

ms : m/z: 221 (MH) $^+$.

anal: Found: C, 65.62; H, 7.44; N, 12.84 %

Calc. for $\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_2$: C, 65.46; H, 7.27; N, 12.73 %

(86) Reaction of Bz-Pro-Ser-OMe (29) with Ru(VIII) : Isolation of N-Benzoyl L-Proline Amide (Bz-Pro-NH $_2$, 30):

Bz-Pro-NH $_2$ (30): (86%)

mp. : syrup

ir : ν_{\max} (KBr) cm^{-1} : 3370 (NH), 3180 (NH), 1660 (amide I), 1602, 1562 (amide II).

nmr : δ [$\text{CDCl}_3 + (\text{CD}_3)_2\text{SO}$]: 2.12 (4H, m, Pro $\text{C}^\beta\text{H}_2 + \text{Pro } \text{C}^\gamma\text{H}_2$), 3.59 (2H, m, Pro $\text{C}^\delta\text{H}_2$), 4.71 (1H, m, Pro C^αH), 7.46 (7H, m, $\text{CONH}_2 + \text{aromatic protons}$).

ms : m/z: 219 (MH)⁺.

anal: Found: C, 66.18; H, 6.52; N, 12.93 %

Calc. for $\text{C}_{12}\text{H}_{14}\text{N}_2\text{O}_2$: C, 66.06; H, 6.42; N, 12.84 %

$[\alpha]_D^{30}$: -56.85 (c, 0.35, MeOH).

(87) Reaction of Boc-Arg($\text{N}^{\text{G}}\text{NO}_2$)-Ser-OMe (31) with Ru(VIII) : Isolation of N-^tButyloxycarbonyl ($\text{N}^{\text{G}}\text{NO}_2$)L-Arginine amide (Boc-Arg($\text{N}^{\text{G}}\text{NO}_2$)- NH_2 , 32): Boc-Arg($\text{N}^{\text{G}}\text{NO}_2$)- NH_2 , (32): (91%)

mp. : syrup

ir : ν_{max} (KBr) cm^{-1} : 3324 (br, NH), 1675 (br, amide I), 1625 (amide I), 1595, 1527 (NO_2), 1367 (NO_2).

nmr : δ [$\text{CDCl}_3 + (\text{CD}_3)_2\text{SO}$]: 1.40 (9H, s, Boc $\text{CH}_3 \times 3$), 2.00 (4H, m, Arg $\text{C}^\beta\text{H}_2 + \text{Arg } \text{C}^\gamma\text{H}_2$), 3.34 (2H, m, Arg $\text{C}^\delta\text{H}_2$), 4.06 (1H, m, Arg C^αH), 5.53 (1H, br, Arg NH), 7.00-7.56 (5H, br, $\text{CONH}_2 + \text{guanidino NH} \times 3$).

ms : m/z: 319 (MH)⁺.

anal: Found: C, 41.09; H, 7.23; N, 27.11 %

Calc. for $\text{C}_{11}\text{H}_{22}\text{N}_6\text{O}_5$: C, 41.51; H, 6.92; N, 26.41 %

(88) Reaction of Bz-Pro-Phe-Ser-OMe (33) with Ru(VIII) : Isolation of N-Benzoyl L-Prolyl-L-Phenylalanine Amide (Bz-Pro-Phe- NH_2 , 34):

Bz-Pro-Phe- NH_2 (34): (70%)

mp. : 188-190°C

ir : ν_{max} (KBr) cm^{-1} : 3310 (NH), 3160 (NH), 1675 (amide I), 1655 (amide I), 1615, 1532 (amide II).

nmr : δ [$\text{CDCl}_3 + (\text{CD}_3)_2\text{SO}$]: 1.50-2.33 (4H, m, Pro $\text{C}^\beta\text{H}_2 + \text{Pro } \text{C}^\gamma\text{H}_2$), 2.90-3.80 (4H, m, Pro $\text{C}^\delta\text{H}_2 + \text{Phe } \text{C}^\beta\text{H}_2$), 4.40-4.75 (2H, m, Pro $\text{C}^\alpha\text{H} + \text{Phe } \text{C}^\alpha\text{H}$), 6.70 (1H, brs, Phe NH), 7.00-8.00 (7H, s + m, $\text{CONH}_2 + \text{aromatic protons}$).

anal: Found: C, 69.22; H, 6.27; N, 11.64 %

Calc. for $C_{21}H_{23}N_3O_3$: C, 69.04; H, 6.30; N, 11.51 %

$[\alpha]_D^{30}$: -75.2 (c, 2.0, MeOH).

(89) Reaction of Boc-Ala-Ala-Ser-OMe (36) with Ru(VIII) : Isolation of N-^tButyloxycarbonyl L-Alanyl-L-Alanine Amide (Boc-Ala-Ala-NH₂, 37):

Boc-Ala-Ala-NH₂ (37): (78%)

mp. : 158-159°C (lit.¹⁶⁵ mp. 162-163°C)

ir : ν_{\max} (KBr)cm⁻¹: 3390 (NH), 3350 (NH), 3315 (NH), 3205 (NH), 1685 (amide I), 1645 (amide I), 1540 (amide II).

nmr : δ [CDCl₃ + (CD₃)₂SO]: 1.26 (3H, d, J=6.5 Hz, Ala CH₃), 1.37 (3H, d, J=6.5 Hz, Ala CH₃), 1.43 (9H, s, Boc CH₃x3), 3.87-4.59 (2H, m, Ala C ^{α} Hx2), 6.10-7.20 (3H, m, Ala NH(Boc) + CONH₂), 7.53 (1H, d, J=7.5 Hz, Ala NH).

anal: Found: C, 50.84; H, 7.93; N, 16.64 %

Calc. for $C_{11}H_{21}N_3O_4$: C, 50.97; H, 8.11; N, 16.22 %

$[\alpha]_D^{30}$: -38.9 (c, 1.7, MeOH).

(90) Reaction of Bz-Val-Phe-Ser-OMe (38) with Ru(VIII) : Isolation of N-Benzoyl L-Valyl-L-Phenylalanine Amide (Bz-Val-Phe-NH₂, 39):

Bz-Val-Phe-NH₂ (39): (65%)

mp. : 238-239°C

ir : ν_{\max} (KBr)cm⁻¹: 3423 (NH), 3318 (NH), 3275 (NH), 3215 (NH), 1675 (amide I), 1655 (amide I), 1632 (amide I), 1618, 1580 (amide II), 1540 (amide II).

anal: Found: C, 68.38; H, 7.16; N, 11.29 %

Calc. for $C_{21}H_{25}N_3O_3$: C, 68.66; H, 6.81; N, 11.44 %

$[\alpha]_D^{30}$: -26.8 (c, 0.9, MeOH).

(91) Reaction of Bz-Thr-OMe (40) with Ru(VIII) : Isolation of Benzamide (2):

Benzamide (2): (84%), please refer to experiment No. 71 for data.

- (92) Reaction of Bz-Gly-Thr-OMe (41) with Ru(VIII) : Isolation of Bz-Gly-NH₂ (8):

Bz-Gly-NH₂ (8): (72%), please refer to experiment No. 75 for data.

- (93) Reaction of Bz-Ala-Thr-OMe (42) with Ru(VIII) : Isolation of Bz-Ala-NH₂ (10):

Bz-Ala-NH₂ (10): (65%), please refer to experiment No. 76 for data.

- (94) Reaction of Bz-Leu-Thr-OMe (43) with Ru(VIII) : Isolation of Bz-Leu-NH₂ (12):

Bz-Leu-NH₂ (12): (72%), please refer to experiment No. 77 for data.

- (95) Reaction of Bz-Phe-Thr-OMe (44) with Ru(VIII) : Isolation of Bz-Phe-NH₂ (14):

Bz-Phe-NH₂ (14): (68%), please refer to experiment No. 78 for data.

- (96) Reaction of Bz-Gly-Phe-Thr-OMe (45) with Ru(VIII) : Isolation of N-Benzoyl Glycyl-L-Phenylalanine Amide (Bz-Gly-Phe-NH₂, 46):

Bz-Gly-Phe-NH₂ (46): (51%)

mp. : 177-178°C

ir : ν_{\max} (KBr) cm⁻¹: 3435 (NH), 3380 (NH), 3285 (NH), 3210 (NH), 1682 (amide I), 1670 (amide I), 1640 (amide I), 1607, 1580 (amide II), 1540 (amide II), 1492.

nmr : δ [CDCl₃ + (CD₃)₂SO]: 3.03 (2H, m, Phe C ^{β} H₂), 3.87 (2H, m, Gly CH₂), 4.60 (1H, m, Phe C ^{α} H), 6.60-8.03 (13H, s+m, Phe NH + CONH₂ + aromatic protons), 8.50 (1H, t, Gly NH).

anal: Found: C, 66.11; H, 5.75; N, 12.74 %

Calc. for C₁₈H₁₉N₃O₃: C, 66.46; H, 5.85; N, 12.92 %

[α]_D³⁰: +2.6 (c, 2.6, MeOH).

- (97) Reaction of Bz-Val-Phe-Thr-OMe (47) with Ru(VIII) : Isolation of Bz-Val-Phe-NH₂ (39):

Bz-Val-Phe-NH₂ (39): (57%), please refer to experiment No. 90 for data.

(98) Reaction of Boc-Ala-Ala-Thr-OMe (48) with Ru(VIII) : Isolation of Boc-Ala-Ala-NH₂ (37):

Boc-Ala-Ala-NH₂ (37): (86%), please refer to experiment No. 89 for data.

(99) Reaction of Z-Ser-Gly-OMe (49) with Ru(VIII) : Isolation of Z-NH[oxalo]-Glycine-Methyl Ester (Z-NH-CO-CO-Gly-OMe, 50):

Z-NH-CO-CO-Gly-OMe (50): (91%)

mp. : 132-133°C

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3250 (NH), 3215 (NH), 3185 (NH), 3050, 1778 (CONHCO), 1755 (carbonyl), 1745 (ester), 1698 (carbamate), 1680 (amide I), 1500 (amide II).

nmr : δ (CDCl₃): 3.81 (3H, s, COOCH₃), 4.11 (2H, d, J=6.5 Hz, Gly CH₂), 5.25 (2H, s, Z CH₂), 7.40 (5H, s, aromatic protons), 7.81 (1H, br, exchangeable with D₂O, Gly NH), 9.37 (1H, brs, exchangeable, CONHCO).

¹³C nmr : δ (100 MHz, CDCl₃): 41.4 (Gly CH₂), 52.6 (OCH₃), 68.4 (OCH₂), 128.5, 128.7, 134.5 (Ph), 149.7 (Z CO), 157.0, 158.3 (-COCO-), 168.5 (Gly-CO).

ms : m/z: 294 (M)⁺.

anal: Found: C, 52.78; H, 4.63; N, 9.26 %

Calc. for C₁₃H₁₄N₂O₆: C, 53.06; H, 4.76; N, 9.52 %

(100) Reaction of Z-Ser-Ala-OMe (51) with Ru(VIII) : Isolation of Z-NH[oxalo]-Alanine-Methyl Ester (Z-NH-CO-CO-Ala-OMe, 52):

Z-NH-CO-CO-Ala-OMe (52): (66%)

mp. : 70-71°C

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3332 (NH), 3254 (NH), 1792 (CONHCO carbonyl), 1749 (ester), 1691 (amide I), 1478 (br, amide II).

nmr : δ (CDCl₃): 1.46 (3H, d, J=6.5 Hz, Ala CH₃), 3.75 (3H, s, COOCH₃), 4.53 (1H, m, Ala C^αH), 5.22 (2H, s, Z CH₂), 7.34

(5H, s, aromatic protons), 7.87 (1H, brd, exchangeable with D₂O, Ala NH), 9.43 (1H, brs, exchangeable, CONHCO).

¹³C nmr : δ (100 MHz, CDCl₃): 17.8 (Ala CH₃), 48.7 (OCH₃), 52.7 (Ala C ^{α} H), 68.3 (OCH₂), 128.5, 128.7, 134.6 (Ph), 149.7 (Z CO), 157.1, 157.5 (-COCO-), 171.5 (Ala-CO).

ms : m/z: 309 (MH)⁺.

anal: Found: C, 54.74; H, 5.46; N, 8.78 %

Calc. for C₁₄H₁₆N₂O₆: C, 54.55; H, 5.19; N, 9.09 %

(101) Reaction of Z-Ser-Phe-OMe (53) with Ru(VIII) : Isolation of Z-NH[oxalo]-Phenylalanine-Methyl Ester (Z-NH-CO-CO-Phe-OMe, 54):

Z-NH-CO-CO-Phe-OMe (54): (82%)

mp. : 82-83°C

ir : ν_{\max} (KBr)cm⁻¹: 3318 (NH), 1779 (CONHCO), 1740 (ester), 1718 (carbamate), 1677 (amide I), 1474 (br, amide II).

nmr : δ (60 MHz, CDCl₃): 3.15 (2H, d, J=6.0 Hz, Phe C ^{β} H₂), 3.70 (3H, s, COOCH₃), 4.76 (1H, m, Phe C ^{α} H), 5.18 (2H, s, Z CH₂), 6.09-7.50 (10H, s + m, aromatic protons), 7.73 (1H, brd, exchangeable with D₂O, Phe NH), 9.26 (1H, brs, exchangeable, CONHCO).

anal: Found: C, 62.17; H, 5.05; N, 6.93 %

Calc. for C₂₀H₂₀N₂O₆: C, 62.50; H, 5.21; N, 7.29 %

[α]_D²⁵: +32.0 (c, 3.2, CHCl₃).

(102) Reaction of Z-Ser-Leu-OMe (55) with Ru(VIII) : Isolation of Z-NH[oxalo]-Leucine-Methyl Ester (Z-NH-CO-CO-Leu-OMe, 56):

Z-NH-CO-CO-Leu-OMe (56): (85%)

mp. : syrup

ir : ν_{\max} (neat)cm⁻¹: 3321 (br, NH), 1793 (CONHCO carbonyl), 1743 (ester), 1690 (br, amide I), 1487 (br, amide II).

nmr : δ (CDCl₃): 0.87 (6H, d, J=5.0 Hz, Leu CH₃x2), 1.62 (3H, m, Leu C ^{β} H₂ + Leu C ^{γ} H), 3.71 (3H, s, COOCH₃), 4.53 (1H, m, Leu

$C^{\alpha}H$), 5.20 (2H, s, Z CH_2), 7.34 (5H, s, aromatic protons),
7.75 (1H, d, $J=7.5$ Hz, Leu NH), 9.43 (1H, brs, CONHCO).

ms : m/z : 350 (M)⁺.

anal: Found: C, 58.73; H, 6.48; N, 8.36 %

Calc. for $C_{17}H_{22}N_2O_6$: C, 58.29; H, 6.29; N, 8.00 %

$[\alpha]_D^{25}$: -7.5 (c, 13.5, $CHCl_3$).

(103) Reaction of Z-Ser-Methyl Anthranilate (57) with Ru(VIII) :

Isolation of Z-NH[oxalo]-Methylantranilate (Z-NH-CO-CO-Methyl-anthranilate, 58):

Z-NH-CO-CO-Methylantranilate (58): (96%)

mp. : 125-126°C

ir : ν_{max} (KBr) cm^{-1} : 3302 (NH), 3185 (NH), 1758 (CONHCO carbonyl), 1735 (ester), 1702 (carbamate), 1687 (amide I), 1603, 1588, 1541 (amide II), 1491.

nmr : δ (60 MHz, $CDCl_3$): 3.93 (3H, s, $COOCH_3$), 5.26 (2H, s, Z CH_2), 6.93-7.76 (7H, s + m, anthranilic ring proton x 2 + Z-aromatic protons), 8.00 (2H, m, anthranilic NH + anthranilic ring proton), 8.56 (1H, d, $J=9.0$ Hz, anthranilic ring proton), 9.41 (1H, s, exchangeable with D_2O , CONHCO).

ms : m/z : 356 (M)⁺.

anal: Found: C, 60.89; H, 4.34; N, 7.64 %

Calc. for $C_{18}H_{16}N_2O_6$: C, 60.67; H, 4.49; N, 7.87 %

(104) Reaction of Z-Ser-Tyr-OMe (59) with Ru(VIII) : Isolation of

Z-NH[oxalo]- NH_2 (Z-NH-CO-CONH₂, 60) and Z-NH-CO-CO-Asp(β -OH)-OMe:

(1) Z-NH-CO-CONH₂ (60): This is obtained as neutral component in 38% yield.

mp. : 198-200°C

ir : ν_{max} (KBr) cm^{-1} : 3375 (NH), 3171 (NH), 1776 (CONHCO), 1684 (br, amide I), 1515 (br, amide II).

nmr : δ [$\text{CDCl}_3 + (\text{CD}_3)_2\text{SO}$]: 5.25 (2H, s, Z CH_2), 7.40 (5H, s, aromatic protons), 8.00 (2H, br, exchangeable with D_2O , CONH_2), 10.31 (1H, s, exchangeable, CONHCO).

ms : m/z : 223 (MH)⁺.

- (11) Z-NH-CO-CO-Asp(β -OH)-OMe: It is obtained as acidic component from the bicarbonate triturated aq. solution. The bicarbonate triturated solution was acidified with 2N H_2SO_4 , saturated with solid NaCl, extracted with EtOAc (2x30 mL) and dried (MgSO_4). The combined organic layers were evaporated in vacuo and the residue obtained, directly converted to the methyl ester (Z-NH-CO-CO-Asp(β -OMe)-OMe, 65) (16%) by esterification with ethereal diazomethane.

mp. : syrup

ir : ν_{max} (neat) cm^{-1} : 3333 (NH), 1788 (CONHCO), 1729 (ester), 1687 (br, amide I), 1485 (br, amide II).

nmr : δ (60 MHz, CDCl_3): 2.96 (2H, m, Asp C^βH_2), 3.68, 3.76 (3H, 3H, s, s, $\text{COOCH}_3 \times 2$), 4.80 (1H, m, Asp C^αH), 5.23 (2H, s, Z CH_2), 7.30 (5H, s, aromatic protons), 8.06 (1H, brd, exchangeable with D_2O , Asp NH), 9.30 (1H, s, exchangeable, CONHCO).

ms : m/z : 367 (MH)⁺.

anal: Found: C, 52.43; H, 4.67; N, 7.56 %

Calc. for $\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_8$: C, 52.46; H, 4.92; N, 7.65 %

- (105) Reaction of Z-Ser-Trp-OMe (61) with Ru(VIII) : Isolation of Z-NH-CO- CONH_2 (60) as neutral part and Z-NH-CO-CO-Asp(β -OH)-OMe as acidic portion:

Products were same as the oxidation products of compound (59).

Z-NH-CO-CO-Asp(β -OH)-OMe (6%) was characterised as the methyl ester.

- (106) Reaction of Z-Ser-Pro-OMe (62) with Ru(VIII) : Isolation of Z-NH[oxalo]-Proline-Methyl Ester (Z-NH-CO-CO-Pro-OMe, 63):

Z-NH-CO-CO-Pro-OMe (63): (50%)

mp. : syrup

ir : ν_{\max} (neat) cm^{-1} : 3360 (NH), 3278 (NH), 1790 (CONHCO), 1734 (br, ester), 1652 (amide I), 1560 (amide II).

nmr : δ (CDCl_3): 2.09 (4H, m, Pro C^βH_2 + Pro $\text{C}^\gamma\text{H}_2$), 3.81 (3H, s, COOCH_3), 4.03 (2H, m, Pro $\text{C}^\delta\text{H}_2$), 4.60 (1H, m, Pro C^αH), 5.26 (2H, s, Z CH_2), 7.47 (5H, s, aromatic protons), 9.75 (1H, brs, CONHCO).

ms : m/z: 335 (MH)⁺.

anal: Found: C, 57.76; H, 5.43; N, 8.44 %

Calc. for $\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_6$: C, 57.49; H, 5.39; N, 8.38 %

(107) Reaction of Z-Ser-Asp(β -OMe)-OMe (64) with Ru(VIII) : Isolation of

Z-NH[oxalo]-Asp(β -OMe)-OMe (Z-NH-CO-CO-Asp(β -OMe)-OMe, 65):

Z-NH-CO-CO-Asp(β -OMe)-OMe (65): (85%), please refer to experiment No. 104 for data.

(108) Reaction of Z-Ser-Ser-OMe (66) with Ru(VIII) : Isolation of

Z-NH-CO-CONH₂ (60):

Z-NH-CO-CONH₂ (60): (32%), please refer to experiment No. 104 for data.

(109) Reaction of Z-Ser-Met-OMe (67) with Ru(VIII) : Isolation of Z-NH[ox-

alo]-Met(SO_2)-OMe (Z-NH-CO-CO-Met(SO_2)-OMe, 68):

Z-NH-CO-CO-Met(SO_2)-OMe (68): (88%)

mp. : 180-181°C

ir : ν_{\max} (KBr) cm^{-1} : 3300 (NH), 1785 (CONHCO), 1750 (ester), 1670 (amide I), 1497 (br, amide II), 1375, 1122 (SO_2).

nmr : δ (CDCl_3): 2.44 (2H, m, Met C^βH_2), 2.92 (3H, s, SO_2CH_3), 3.04 (2H, m, Met $\text{C}^\gamma\text{H}_2$), 3.81 (3H, s, COOCH_3), 4.66 (1H, m, Met C^αH), 5.22 (2H, s, Z CH_2), 7.35 (5H, s, aromatic protons), 8.19 (1H, d, J=7.5 Hz, exchangeable with D_2O , Met NH), 9.38 (1H, s, exchangeable, CONHCO).

ms : m/z: 401 (MH)⁺.

(110) Reaction of Z-Ser-Aib-Ser-OMe (69) with Ru(VIII) : Isolation of Z-NH[oxalo]-Aib-NH₂ (Z-NH-CO-CO-Aib-NH₂, 70):

Z-NH-CO-CO-Aib-NH₂ (70): (84%)

mp. : syrup

ir : ν_{\max} (KBr)cm⁻¹: 3486 (NH), 3348 (NH), 1785 (CONHCO), 1687 (br, amide I), 1497 (amide II).

nmr : δ (CDCl₃): 1.62 (6H, brs, Aib CH₃x2), 5.18 (2H, s, Z CH₂), 5.96 (2H, br, CONH₂), 7.33 (5H, s, aromatic protons), 8.03 (1H, s, Aib NH), 9.40 (1H, s, CONHCO).

ms : m/z: 308 (MH)⁺.

anal: Found: C, 54.63; H, 5.88; N, 13.43 %

Calc. for C₁₄H₁₇N₃O₅: C, 54.72; H, 5.54; N, 13.68 %

(111) Reaction of Z-Ser-Leu-Ser-OMe (71) with Ru(VIII) : Isolation of Z-NH[oxalo]-Leucine-Amide (Z-NH-CO-CO-Leu-NH₂, 72):

Z-NH-CO-CO-Leu-NH₂ (72): (50%)

mp. : syrup

ir : ν_{\max} (neat)cm⁻¹: 3392 (NH), 3294 (NH), 3194 (NH), 1783 (CONHCO), 1659 (br, amide I), 1492 (amide II).

nmr : δ (60 MHz, CDCl₃): 0.91 (6H, brd, Leu CH₃x2), 1.63 (3H, m, Leu C ^{β} H₂ + Leu C ^{γ} H), 4.43 (1H, m, Leu C ^{α} H), 5.18 (2H, s, Z CH₂), 6.30 (2H, br, exchangeable with D₂O, CONH₂), 7.31 (5H, s, aromatic protons), 8.05 (1H, d, J=7.5 Hz, exchangeable, Leu NH), 9.50 (1H, s, exchangeable, CONHCO).

ms : m/z: 335 (M)⁺.

anal: Found: C, 57.43; H, 6.48; N, 12.17 %

Calc. for C₁₆H₂₁N₃O₅: C, 57.31; H, 6.27; N, 12.54 %

* Z-Ser-Leu-Ser-OMe (71), when reacted at pH 6, afforded a mixture of Z-NH-CO-CO-Leu-NH₂ (72, 30%) and Z-Ser-Leu-NH₂ (103, 50%).

(112) Reaction of Z-Ser-Gly-Ser-OMe (73) with Ru(VIII) : Isolation of Z-NH[oxalo]-Glycine-Amide (Z-NH-CO-CO-Gly-NH₂, 74):

Z-NH-CO-CO-Gly-NH₂ (74): (83%)

mp. : 204-205°C

ir : ν_{\max} (KBr)cm⁻¹: 3402 (NH), 3208 (NH), 1772 (CONHCO), 1686 (amide I), 1654 (amide I), 1493 (amide II).

nmr : δ [CDCl₃ + (CD₃)₂SO]: 3.80 (2H, d, J=6.5 Hz, Gly CH₂), 5.22 (2H, s, Z CH₂), 6.80-7.42 (7H, m, CONH₂ + aromatic protons), 9.00 (1H, t, exchangeable, Gly NH), 10.75 (1H, s, exchangeable, CONHCO).

ms : m/z: 279 (M)⁺.

anal: Found: C, 51.29; H, 4.38; N, 15.35 %

Calc. for C₁₂H₁₃N₃O₅: C, 51.61; H, 4.66; N, 15.05 %

* Reaction at pH 6 yielded the same product.

(113) Reaction of Z-Ser-Pro-Ser-OMe (75) with Ru(VIII) at pH 6 : Isolation of Z-NH[oxalo]-Proline-Amide (Z-NH-CO-CO-Pro-NH₂, 76) and partially oxidized product Z-Ser-Pro-NH₂ (77):

(i) Z-NH-CO-CO-Pro-NH₂ (76): (40%)

mp. : gummy

ir : ν_{\max} (KBr)cm⁻¹: 3348 (NH), 1785 (CONHCO), 1679 (br, amide I), 1497 (amide II).

nmr : δ (CDCl₃): 2.10 (4H, m, Pro C ^{β} H₂ + Pro C ^{γ} H₂), 3.80 (2H, m, Pro C ^{γ} H₂), 4.50 (1H, m, Pro C ^{α} H), 5.20 (2H, s, Z CH₂), 7.33 (7H, brs, CONH₂ + aromatic protons), 9.80 (1H, s, CONHCO).

ms : m/z: 320 (MH)⁺.

(ii) Z-Ser-Pro-NH₂ (77): (30%)

mp. : 205-206°C

ir : ν_{\max} (KBr)cm⁻¹: 3220, 1672 (br).

nmr : δ (CDCl₃): 2.00 (4H, br, Pro C ^{β} H₂ + Pro C ^{γ} H₂), 3.31-4.03 (4H, m, Pro C ^{δ} H₂ + Ser C ^{β} H₂), 5.09 (4H, s+br, Pro C ^{α} H + Ser

$C^{\alpha}H + Z CH_2$), 6.15 (1H, br, exchangeable, Ser NH),
6.93-7.53 (7H, s+br, $CONH_2$ + aromatic protons).

(114) Reaction of Z-Thr-Gly-OMe (78) with Ru(VIII) : Isolation of Z-NH[oxalo]-Glycine-Methyl Ester (Z-NH-CO-CO-Gly-OMe, 50):

Z-NH-CO-CO-Gly-OMe (50): (83%), please refer to experiment No. 99 for data.

(115) Reaction of Z-Thr-Ala-OMe (79) with Ru(VIII) : Isolation of Z-NH[oxalo]-Alanine-Methyl Ester (Z-NH-CO-CO-Ala-OMe, 52):

Z-NH-CO-CO-Ala-OMe (52): (93%), please refer to experiment No. 100 for data.

(116) Reaction of Z-Thr-Phe-OMe (80) with Ru(VIII) : Isolation of Z-NH[oxalo]-Phenylalanine-Methyl Ester (Z-NH-CO-CO-Phe-OMe, 54):

Z-NH-CO-CO-Phe-OMe (54): (85%), please refer to experiment No. 101 for data.

(117) Reaction of Z-Thr-Leu-OMe (81) with Ru(VIII) : Isolation of Z-NH[oxalo]-Leucine-Methyl Ester (Z-NH-CO-CO-Leu-OMe, 56):

Z-NH-CO-CO-Leu-OMe (56): (86%), please refer to experiment No. 102 for data.

(118) Reaction of Z-Thr-Thr-OMe (82) with Ru(VIII) : Isolation of Z-NH-CO-CO-NH₂ (60):

Z-NH-CO-CO-NH₂ (60): (40%), please refer to experiment No. 104 for data.

(119) Reaction of Z-Thr-Lys($N^{\omega}Z$)-OMe (83) with Ru(VIII) : Isolation of Z-NH[oxalo]-Lysine($N^{\omega}Z$)-Methyl Ester (Z-NH-CO-CO-Lys($N^{\omega}Z$)-OMe, 84):

Z-NH-CO-CO-Lys($N^{\omega}Z$)-OMe (84): (60%)

mp. : syrup

ir : ν_{\max} (KBr) cm^{-1} : 3329 (NH), 1791 (CONHCO), 1700 (br, amide I), 1494 (br, amide II).

nmr : δ (CDCl₃): 1.03-1.93 (6H, m, Lys $C^{\beta}H_2$ + Lys $C^{\gamma}H_2$ + Lys $C^{\delta}H_2$), 3.12 (2H, m, Lys $C^{\omega}H_2$), 3.68 (3H, s, COOCH₃), 4.46

(1H, m, Lys C^αH), 5.09 (4H, s, Z CH₂x2), 7.34 (11H, brs + m, Lys N^ωH + aromatic protons), 7.90 (1H, d, J=7.5 Hz, exchangeable with D₂O, Lys N^αH), 9.43 (1H, brs, exchangeable, CONHCO).

anal: Found: C, 59.87; H, 5.82; N, 8.37 %

Calc. for C₂₅H₂₉N₃O₈: C, 60.12; H, 5.81; N, 8.42 %

(120) Reaction of Boc-Thr-Ala-Ala-OMe (85) with Ru(VIII) : Isolation of Boc-NH[oxalo]-Alanyl-Alanine-Methyl Ester (Boc-NH-CO-CO-Ala-Ala-OMe, 86):

Boc-NH-CO-CO-Ala-Ala-OMe (86): (92%)

mp. : syrup

ir : ν_{\max} (KBr)cm⁻¹: 3394 (NH), 3302 (NH), 1786 (CONHCO), 1746 (ester), 1660 (br, amide I), 1542 (amide II).

nmr : δ (CDCl₃): 1.00-1.62 (15H, m, Boc CH₃x3 + Ala CH₃x2), 3.79 (3H, s, COOCH₃), 4.54 (2H, m, Ala C^αHx2), 6.73 (1H, d, J=7.5 Hz, Ala NH), 8.06 (1H, d, J=7.5 Hz, Ala NH), 9.28 (1H, s, CONHCO).

anal: Found: C, 48.53; H, 6.44; N, 12.27 %

Calc. for C₁₄H₂₃N₃O₇: C, 48.70; H, 6.67; N, 12.17 %

(121) Reaction of Z-Thr-Ala-Ala-OMe (87) with Ru(VIII) : Isolation of Z-NH[oxalo]-Alanyl-Alanine-Methyl Ester (Z-NH-CO-CO-Ala-Ala-OMe, 88):

Z-NH-CO-CO-Ala-Ala-OMe (88): (76%)

mp. : syrup

ir : ν_{\max} (KBr)cm⁻¹: 3328 (NH), 1793 (CONHCO), 1742 (ester), 1689 (amide I), 1487 (br, amide II).

nmr : δ (CDCl₃): 1.46 (6H, d, J=6.5 Hz, Ala CH₃x2), 3.78 (3H, s, COOCH₃), 4.56 (2H, m, Ala C^αHx2), 5.25 (2H, s, Z CH₂), 7.37 (6H, s, Ala NH + aromatic protons), 7.93 (1H, brd, Ala NH), 9.46 (1H, brs, CONHCO).

anal: Found: C, 53.47; H, 5.18; N, 11.33 %

Calc. for $C_{17}H_{21}N_3O_7$: C, 53.83; H, 5.54; N, 11.08 %

(122) Reaction of Z-Thr-Cys(S-Bzl)-OMe (89) with Ru(VIII) : Isolation of Z-NH[oxalo]-Cysteine(SO₂-Bzl)-Methyl Ester (Z-NH-CO-CO-Cys(SO₂-Bzl)-OMe, 90):

Z-NH-CO-CO-Cys(SO₂-Bzl)-OMe (90): (79%)

mp. : 101-102°C

ir : ν_{\max} (KBr) cm^{-1} : 3302 (NH), 1783 (CONHCO), 1743 (ester), 1673 (amide I), 1483 (br, amide II), 1311, 1173, 1134 (SO₂).

nmr : δ (CDCl₃): 3.40 (2H, d, J=5.0 Hz, Cys C ^{β} H₂), 3.78 (3H, s, COOCH₃), 4.28 (2H, s, Bzl CH₂), 4.96 (1H, m, Cys C ^{α} H), 5.25 (2H, s, Z CH₂), 7.43 (10H, s, aromatic protons), 8.40 (1H, d, J=7.5 Hz, exchangeable with D₂O, Cys NH), 9.37 (1H, s, exchangeable, CONHCO).

ms : m/z: 463 (MH)⁺.

anal: Found: C, 54.18; H, 4.68; N, 5.83 %

Calc. for $C_{21}H_{22}N_2O_8S$: C, 54.55; H, 4.76; N, 6.06 %

$[\alpha]_D^{25}$: +1.82 (c, 1.7, CHCl₃).

(123) Reaction of Z-Thr-Leu-Leu-OMe (91) with Ru(VIII) : Isolation of Z-NH[oxalo]-Leucyl-Leucine-Methyl Ester (Z-NH-CO-CO-Leu-Leu-OMe, 92):

Z-NH-CO-CO-Leu-Leu-OMe (92): (96%)

mp. : syrup

ir : ν_{\max} (neat) cm^{-1} : 3308 (NH), 1788 (CONHCO), 1744 (ester), 1665 (br, amide I), 1535 (amide II), 1484.

nmr : δ (CDCl₃): 0.93 (12H, brs, Leu CH₃x4), 1.62 (6H, m, Leu C ^{β} H₂x2 + Leu C ^{γ} Hx2), 3.72 (3H, s, COOCH₃), 4.56 (2H, m, Leu C ^{α} Hx2), 5.22 (2H, s, Z CH₂), 6.78 (1H, d, J=7.5 Hz, exchangeable with D₂O, Leu NH), 7.37 (5H, s, aromatic protons), 8.00 (1H, d, J=7.5 Hz, exchangeable, Leu NH), 9.62 (1H, s, exchangeable, CONHCO).

ms : m/z: 464 (MH)⁺.

anal: Found: C, 59.44; H, 7.23; N, 9.27 %

Calc. for $C_{23}H_{33}N_3O_7$: C, 59.61; H, 7.13; N, 9.07 %

(124) Reaction of Z-Thr-Leu-Leu-Leu-OMe (93) with Ru(VIII) : Isolation of Z-NH[oxalo]-Leucyl-Leucyl-Leucine-Methyl Ester (Z-NH-CO-CO-Leu-Leu-Leu-OMe, 94):

Z-NH-CO-CO-Leu-Leu-Leu-OMe (94): (36%)

mp. : syrup

ir : ν_{\max} (neat) cm^{-1} : 3289 (NH), 1784 (CONHCO), 1742 (ester), 1651 (br, amide I), 1484 (br, amide II).

nmr : δ (300 MHz, CDCl_3): 0.9 (18H, brs, Leu $\text{CH}_3 \times 6$), 1.66 (9H, m, Leu $\text{C}^\beta \text{H}_2 \times 3$ + Leu $\text{C}^\gamma \text{H} \times 3$), 3.72 (3H, s, COOCH_3), 4.34-4.64 (3H, m, Leu $\text{C}^\alpha \text{H} \times 3$), 5.24 (2H, s, Z CH_2), 6.40 (2H, m, exchangeable with D_2O , Leu $\text{NH} \times 2$), 7.36 (5H, s, aromatic protons), 7.80 (1H, d, $J=7.5$ Hz, exchangeable, Leu NH), 9.40 (1H, s, exchangeable, CONHCO).

ms : m/z : 577 (MH)⁺.

anal: Found: C, 60.09; H, 7.73; N, 10.04 %

Calc. for $C_{29}H_{44}N_4O_8$: C, 60.42; H, 7.64; N, 9.72 %

(125) Reaction of Bz-Ser-NH₂ (95) with Ru(VIII) : Isolation of Bz-NH[oxalo]-NH₂ (Bz-NH-CO-CONH₂, 96):

Bz-NH-CO-CONH₂ (96): (91%)

mp. : 111-112°C

ir : ν_{\max} (KBr) cm^{-1} : 3428 (NH), 3376 (NH), 3324 (NH), 3283 (NH), 3172 (NH), 1763 (CONHCO), 1702, 1678 (amide I), 1646 (amide I), 1597, 1578 (amide II), 1500.

nmr : δ (CDCl_3): 6.36 (2H, brd, exchangeable with D_2O , CONH₂), 7.15-8.12 (5H, m, aromatic protons), 10.56 (1H, s, exchangeable with D_2O , CONHCO).

ms : m/z : 193 (MH)⁺.

anal: Found: C, 56.08; H, 4.26; N, 14.96 %

Calc. for $C_9H_8N_2O_3$: C, 56.25; H, 4.17; N, 14.58 %

(126) Reaction of Z-Ser-NH₂ (97) with Ru(VIII) : Isolation of Z-NH-CO-CONH₂ (60):

Z-NH-CO-CONH₂ (60): (65%), please refer to experiment No. 104 for data.

(127) Reaction of Z-Leu-Ser-NH₂ (98) with Ru(VIII) : Isolation of Z-Leucyl-NH[oxalo]-Amide (Z-Leu-NH-CO-CONH₂, 99):

Z-Leu-NH-CO-CONH₂ (99): (88%)

mp. : 151-152°C

ir : ν_{\max} (KBr) cm^{-1} : 3396 (NH), 3304 (NH), 1764 (CONHCO), 1704, 1678 (amide I), 1518 (amide II).

nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 0.93 (6H, d, $J=5.0$ Hz, Leu $\text{CH}_3 \times 2$), 1.62 (3H, m, Leu C^βH_2 + Leu C^γH), 4.65 (1H, m, Leu C^αH), 5.18 (2H, s, Z CH_2), 6.71 (1H, d, $J=7.5$ Hz, exchangeable with D_2O , Leu NH), 7.27-7.75 (7H, s+br, CONH₂ + aromatic protons), 10.50 (1H, s, exchangeable, CONHCO).

ms : m/z : 336 (MH)⁺.

anal: Found: C, 57.13; H, 6.48; N, 12.67 %

Calc. for $C_{16}H_{21}N_3O_5$: C, 57.31; H, 6.27; N, 12.54 %

$[\alpha]_D^{26}$: -20.94 (c, 0.85, MeOH).

(128) Reaction of Bz-Gly-Ser-NH₂ (100) with Ru(VIII) : Isolation of Bz-Glycyl-NH[oxalo]-Amide (Bz-Gly-NH-CO-CONH₂, 101):

Bz-Gly-NH-CO-CONH₂ (101): (90%)

mp. : 164-165°C

ir : ν_{\max} (KBr) cm^{-1} : 3397 (NH), 3288 (NH), 3160 (NH), 1780 (CONHCO), 1687 (amide I), 1637 (amide I), 1557 (amide II), 1490.

nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 4.40 (2H, d, $J=5.0$ Hz, Gly CH_2), 6.71 (1H, br, exchangeable with D_2O , Gly NH), 7.00-8.46 (7H, m, CONH₂ + aromatic protons), 10.65 (1H, s,

exchangeable, CONHCO).

ms : m/z : 250 (MH)⁺.

anal: Found: C, 52.81; H, 4.46; N, 17.16 %

Calc. for $C_{11}H_{11}N_3O_4$: C, 53.01; H, 4.42; N, 16.87 %

(129) Reaction of Z-Thr-NH₂ (102) with Ru(VIII) : Isolation of Z-NH-CO-CO-NH₂ (60):

Z-NH-CO-CO-NH₂ (60): (61%), please refer to experiment No. 104 for data.

(130) Reaction of Z-Ser-Leu-NH₂ (103) with Ru(VIII) : Isolation of Z-NH-CO-CO-Leu-NH₂ (72):

Z-NH-CO-CO-Leu-NH₂ (72): (80%), please refer to experiment No. 111 for data.

(131) Reaction of Z-Ser-Ser-NH₂ (104) with Ru(VIII) : Isolation of Z-NH-CO-CO-NH₂ (60).

Z-NH-CO-CONH₂ (60): (64%), please refer to experiment No. 104 for data.

(132) Reaction of Bz-Leu-Ser-Leu-OMe (105) with Ru(VIII) : Isolation of Bz-Leucyl-NH[oxalo]-Leucine-Methyl Ester (Bz-Leu-NH-CO-CO-Leu-OMe, 106):

Bz-Leu-NH-CO-CO-Leu-OMe (106): (55%)

mp. : 75-77°C

ir : ν_{\max} (KBr) cm⁻¹: 3330 (NH), 1770 (CONHCO), 1745 (ester), 1690 (amide I), 1645 (amide I), 1605, 1578 (amide II), 1530 (amide II), 1470.

nmr : δ (CDCl₃): 0.93 (12H, d, J=5.0 Hz, Leu CH₃x4), 1.68 (6H, m, Leu C ^{β} H₂x2 + Leu C ^{γ} Hx2), 3.75 (3H, s, COOCH₃), 4.59 (1H, m, Leu C ^{α} H), 5.34 (1H, m, Leu C ^{α} H), 7.04 (1H, d, J=7.5 Hz, exchangeable with D₂O, Leu NH), 7.25-8.03 (6H, m, Leu NH + aromatic protons), 10.23 (1H, s, exchangeable, CONHCO).

anal: Found: C, 61.28; H, 7.18; N, 9.36 %

Calc. for $C_{22}H_{31}N_3O_6$: C, 60.97; H, 7.16; N, 9.70 %

(133) Reaction of Bz-Ala-Ser-Ala-OMe (107) with Ru(VIII) : Isolation of Bz-Alanyl-NH[oxalo]-Alanine-Methyl Ester (Bz-Ala-NH-CO-CO-Ala-OMe, 108):

Bz-Ala-NH-CO-CO-Ala-OMe (108): (98%)

mp. : 140-141°C

ir : ν_{\max} (KBr) cm^{-1} : 3277 (NH), 1762 (CONHCO), 1740 (ester), 1674 (amide I), 1630 (amide I), 1534 (amide II), 1488.

nmr : δ (CDCl_3): 1.46, 1.51 (3H, 3H, d, d, $J=6.5$ Hz, 6.5 Hz, Ala $\text{CH}_3 \times 2$), 3.78 (3H, s, COOCH_3), 4.53 (1H, m, Ala C^αH), 5.31 (1H, m, Ala C^αH), 6.81 (1H, d, $J=7.5$ Hz, exchangeable with D_2O , Ala NH), 7.28-8.00 (6H, m, Ala NH + aromatic protons), 10.09 (1H, s, exchangeable, CONHCO).

ms : m/z : 349 (M)⁺.

anal: Found: C, 55.43; H, 5.08; N, 11.87 %

Calc. for $C_{16}H_{19}N_3O_6$: C, 55.01; H, 5.44; N, 12.03 %

$[\alpha]_D^{28}$: +1.2 (c, 1.6, CHCl_3).

(134) Reaction of Z-Leu-Ser-His-OMe (109) with Ru(VIII) : Isolation of Z-Leucyl-NH[oxalo]-Asparagine(N-formyl)-Methyl Ester (Z-Leu-NH-CO-CO-NH-CH(CO_2Me)- CH_2 -CO-NH-CHO, 110):

Z-Leu-NH-CO-CO-NH-CH(CO_2Me)- CH_2 -CO-NH-CHO (110): (73%), pale yellow crystals from EtOAc/hexane.

mp. : 79-85°C

ir : ν_{\max} (KBr) cm^{-1} : 3396 (NH), 3319 (NH), 3209 (NH), 1775 (CONHCO), 1732 (ester), 1660 (amide I), 1543 (amide II).

nmr : δ (CDCl_3): 0.90 (6H, br, Leu $\text{CH}_3 \times 2$), 1.57 (3H, m, Leu C^βH_2 + Leu C^γH), 3.14 (2H, m, C^βH_2 of N-formyl asparagine), 3.78 (3H, s, COOCH_3), 4.87 (2H, m, Leu C^αH + C^αH of N-formyl asparagine), 5.10 (2H, s, Z CH_2), 5.50 (1H, br, exchangeable with D_2O , Leu NH), 7.34 (5H, s, aromatic

protons), 8.40 (1H, d, $J=7.5$ Hz, exchangeable with D_2O , N-formyl asparagine $N^{\alpha}H$), 9.12 (1H, d, non-exchangeable, CHO), 10.18 (1H, s, exchangeable, CONHCO), 10.43 (1H, d, $J=7.5$ Hz, exchangeable, $NHCHO$).

Attempted purification of compound (110) on silica gel by preparative tlc, yielded the cleaved product Z-Leu- NH_2 (111) (~90%).

(135) Reaction of Z-Gly-Ser-Gly-OMe (112) with Ru(VIII) : Isolation of Z-Glycyl-NH[oxalo]-Glycine-Methyl Ester (Z-Gly-NH-CO-CO-Gly-OMe, 113):

Z-Gly-NH-CO-CO-Gly-OMe (113): (50%)

mp. : 115-116°C

ir : ν_{\max} (KBr) cm^{-1} : 3383 (NH), 3325 (NH), 3272 (NH), 3210 (NH), 3169 (NH), 1760 (CONHCO), 1720 (ester), 1690 (br, carbamate), 1651 (amide I), 1531 (amide II), 1512 (amide II), 1493.

nmr : δ [$CDCl_3$ + $(CD_3)_2SO$]: 3.75 (3H, s, $COOCH_3$), 4.06, 4.34 (2H, 2H, d, d, $J=5.0$ Hz, 5.0 Hz, Gly $CH_2 \times 2$), 5.12 (2H, s, Z CH_2), 6.46 (1H, br, exchangeable with D_2O , Gly $NH(Z)$), 7.34 (5H, s, aromatic protons), 8.75 (1H, brm, exchangeable, Gly NH), 10.25 (1H, s, exchangeable, CONHCO).

ms : m/z : 351 (M)⁺.

anal: Found: C, 50.87; H, 4.64; N, 11.79 %

Calc. for $C_{15}H_{17}N_3O_7$: C, 51.28; H, 4.84; N, 11.97 %

(136) Reaction of Bz-Pro-Ser-Pro-OMe (114) with Ru(VIII) : Isolation of Bz-Prolyl-NH[oxalo]-Proline-Methyl ester (Bz-Pro-NH-CO-CO-Pro-OMe, 115):

Bz-Pro-NH-CO-CO-Pro-OMe (115): (40%)

mp. : syrup

ir : ν_{\max} (KBr) cm^{-1} : 3320 (br, NH), 1725 (ester), 1640 (amide I), 1600, 1560 (amide II).

nmr : δ (CDCl_3): 1.75-2.31 (8H, m, Pro $\text{C}^\beta\text{H}_2 \times 2$ + Pro $\text{C}^\gamma\text{H}_2 \times 2$), 3.59-3.93 (7H, s+m, COOCH_3 + Pro $\text{C}^\gamma\text{H}_2 \times 2$), 4.25-4.93 (2H, m, Pro $\text{C}^\alpha\text{H} \times 2$), 6.18 (1H, br, exchangeable with D_2O , CONHCO), 7.28-8.00 (5H, m, aromatic protons).

$[\alpha]_D^{28}$: -38.18 (c, 0.27, CHCl_3).

(137) Reaction of Bz-Aib-Ser-Aib-OMe (116) with Ru(VIII) : Isolation of Bz-Aib-NH-CO-CO-Aib-OMe (117):

Bz-Aib-NH-CO-CO-Aib-OMe (117): (86%)

mp. : 173-174°C

ir : ν_{max} (KBr) cm^{-1} : 3377 (NH), 3326 (NH), 1770 (CONHCO), 1738 (ester), 1702, 1680 (amide I), 1657 (amide I), 1519 (amide II).

nmr : δ (CDCl_3): 1.66 (12H, s, s, Aib $\text{CH}_3 \times 2$; $\text{CH}_3 \times 2$), 3.71 (3H, s, COOCH_3), 6.78 (1H, s, Aib NH(Bz)), 7.25-8.00 (6H, m, Aib NH + aromatic protons), 10.68 (1H, s, CONHCO).

ms : m/z: 378 (MH)⁺.

anal: Found: C, 57.46; H, 6.27; N, 11.26 %

Calc. for $\text{C}_{18}\text{H}_{23}\text{N}_3\text{O}_6$: C, 57.29; H, 6.10; N, 11.14 %

(138) Reaction of Z-Leu-Ser-Ser-Leu-Leu-Ser-Leu-OMe (119) with Ru(VIII):

Isolation of Z-Leu-NH₂ (111):

Z-Leu-NH₂ (111): This is the sole product which could be isolated from the reaction mixture.

mp. : 117-118°C (lit.¹⁶⁶ mp. 125-126°C)

ir : ν_{max} (KBr) cm^{-1} : 3391 (NH), 3321 (NH), 3191 (NH), 1657 (amide I), 1543 (amide II).

nmr : δ (CDCl_3): 0.90 (6H, d, J=5.0 Hz, Leu $\text{CH}_3 \times 2$), 1.62 (3H, m, Leu C^βH_2 + Leu C^γH), 4.18 (1H, m, Leu C^αH), 5.09 (2H, s, Z CH_2), 5.31 (1H, d, J=7.5 Hz, exchangeable with D_2O , Leu NH), 5.90 (2H, br, exchangeable, CONH_2), 7.34 (5H, s, aromatic protons).

anal: Found: C, 63.43; H, 7.29; N, 10.43 %

Calc. for $C_{14}H_{20}N_2O_3$: C, 63.64; H, 7.58; N, 10.61 %

(139) Reaction of Bz-Ala-Thr-Ala-OMe (120) with Ru(VIII) : Isolation of Bz-Ala-NH-CO-CO-Ala-OMe (108):

Bz-Ala-NH-CO-CO-Ala-OMe (108): (80%), please refer to experiment No. 133 for data.

(140) Reaction of Bz-Pro-Thr-Pro-OMe (121) with Ru(VIII) : Isolation of Bz-Pro-NH-CO-CO-Pro-OMe (115):

Bz-Pro-NH-CO-CO-Pro-OMe (115): (69%), please refer to experiment No. 136 for data.

(141) Reaction of Bz-Ala-Ala-Thr-Ala-Ala-OMe (122) with Ru(VIII) : Isolation of Bz-Alanyl-Alanyl-NH[oxalo]-Alanyl-Alanine-Methyl Ester (Bz-Ala-Ala-NH-CO-CO-Ala-Ala-OMe, 123):

Bz-Ala-Ala-NH-CO-CO-Ala-Ala-OMe (123): (20%)

mp. : gummy

ir : ν_{\max} (KBr) cm^{-1} : 3295 (br, NH), 1756 (br, CONHCO, ester), 1637 (amide I), 1548 (amide II).

nmr : δ (CDCl_3): 1.40 (12H, d, $J=6.5$ Hz, Ala $\text{CH}_3 \times 4$), 3.78 (3H, s, COOCH_3), 4.59 (4H, m, Ala $\text{C}^\alpha\text{H} \times 4$), 6.43-8.28 (10H, m, Ala $\text{NH} \times 4$ + CONHCO + aromatic protons).

ms : m/z : 492 (MH) $^+$.

$[\alpha]_D^{28}$: -18.97 (c, 1.66, MeOH).

The following typical procedure was used for the preparation of the core element, $\text{MeO-A}_{aa}\text{-CO-CO-A}_{aa}\text{-OMe}$.

A solution of oxalyl chloride (0.093 mL, 1 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise to a well stirred solution of $\text{A}_{aa}\text{-OMe}$ hydrochloride (2 mmol) in dry CH_2Cl_2 (30 mL) containing triethylamine (0.7 mL, 5 mmol) at 0°C over 0.5 h. After 12 h of stirring at room temperature, the reaction mixture was washed with 5% NaHCO_3 , dried (MgSO_4) and evaporated in vacuo. The residue obtained was crystallized from methanol or ethyl acetate to give pure oxalo peptides.

(142) N,N'-Oxalo bis(Glycine) Dimethyl Ester ($\text{MeO-Gly-CO-CO-Gly-OMe}$, 124):

$\text{MeO-Gly-CO-CO-Gly-OMe}$ (124): (86%)

mp. : crystals from methanol, $120\text{--}121^\circ\text{C}$ (lit.¹⁷¹ mp. $159\text{--}160^\circ\text{C}$)

ir : ν_{max} (KBr) cm^{-1} : 3373 (NH), 1737 (ester), 1680 (amide I), 1507 (amide II), 1443, 1406, 1372.

nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 3.73 (6H, s, $\text{COOCH}_3 \times 2$), 4.00 (4H, d, $J=6.0$ Hz, Gly $\text{CH}_2 \times 2$), 8.82 (2H, t, exchangeable with D_2O , Gly $\text{NH} \times 2$).

ms : m/z : 233 (MH^+), 117 ($\text{M}/2 + \text{H}^+$).

anal: Found: C, 41.46; H, 5.18; N, 12.33 %

Calc. for $\text{C}_8\text{H}_{12}\text{N}_2\text{O}_6$: C, 41.38; H, 5.17; N, 12.07 %

(143) N,N'-Oxalo bis(Alanine) Dimethyl Ester ($\text{MeO-Ala-CO-CO-Ala-OMe}$, 125):

$\text{MeO-Ala-CO-CO-Ala-OMe}$ (125): (55%)

mp. : crystals form EtOAc/hexane, $156\text{--}60^\circ\text{C}$ (lit.¹⁷³ mp. $166\text{--}167^\circ\text{C}$)

ir : ν_{max} (KBr) cm^{-1} : 3279 (NH), 1737 (ester), 1659 (amide I), 1531 (amide II), 1449.

nmr : δ (60 MHz, CDCl_3): 1.50 (6H, d, $J=6.5$ Hz, Ala $\text{CH}_3 \times 2$), 3.76 (6H, s, $\text{COOCH}_3 \times 2$), 4.50 (2H, m, Ala $\text{C}^\alpha\text{H} \times 2$), 7.76 (2H, br,

exchangeable with D₂O, Ala NHx2).

361

ms : m/z: 261 (MH)⁺.

anal: Found: C, 45.86; H, 6.48; N, 11.77 %

Calc. for C₁₀H₁₆N₂O₆: C, 46.15; H, 6.15; N, 10.77 %

[α]_D²⁴: -73.45 (c, 0.55, MeOH).

(144) N,N'-Oxalo bis(Phenylalanine) Dimethyl Ester (MeO-Phe-CO-CO-Phe-OMe, 126):

MeO-Phe-CO-CO-Phe-OMe (126): (60%)

mp. : 192-193°C

ir : ν_{max} (KBr)cm⁻¹: 3284 (NH), 1738 (ester), 1661 (amide I), 1523 (amide II), 1440.

nmr : δ (CDCl₃): 3.15 (4H, d, J=6.25 Hz, Phe C^βH₂x2), 3.73 (6H, s, COOCH₃x2), 4.80 (2H, m, Phe C^αHx2), 7.31 (10H, s, aromatic protons), 7.75 (2H, d, J=7.5 Hz, exchangeable, Phe NHx2).

ms : m/z: 413 (MH)⁺, 206 (M/2)⁺.

anal: Found: C, 64.17; H, 5.71; N, 6.96 %

Calc. for C₂₂H₂₄N₂O₆: C, 64.08; H, 5.83; N, 6.80 %

[α]_D²⁴: +28.00 (c, 0.10, MeOH).

(145) N,N'-Oxalo bis(Leucine) Dimethyl Ester (MeO-Leu-CO-CO-Leu-OMe, 127):

MeO-Leu-CO-CO-Leu-OMe (127): (88%)

mp. : crystals from methanol, 114-145°C

ir : ν_{max} (KBr)cm⁻¹: 3345 (NH), 3299 (NH), 1741 (ester), 1664 (amide I), 1516 (amide II), 1437.

nmr : δ (CDCl₃): 0.93 (12H, d, J=5.0 Hz, Leu CH₃x4), 1.64 (6H, m, Leu C^βH₂x2 + Leu C^γHx2), 3.73 (6H, s, COOCH₃x2), 4.57 (2H, m, Leu C^αHx2), 7.73 (2H, d, J=7.5 Hz, exchangeable, Leu NHx2).

ms : m/z: 345 (MH)⁺, 172 (M/2)⁺.

anal: Found: C, 56.14; H, 8.22; N, 8.48 %

I), 1500 (amide II), 1432.

nmr : δ (CDCl_3): 3.84, 3.90 (3H, 3H, s, s, $\text{COOCH}_3 \times 2$), 4.00 (2H, m, Ser C^βH_2), 4.65 (1H, m, Ser C^αH), 6.09, 6.68 (1H, 1H, s, s, $=\text{CH}_2$), 8.28 (1H, d, $J=7.5$ Hz, exchangeable, Ser NH), 9.56 (1H, s, exchangeable, Δ Ala NH).

ms : m/z : 275 (MH)⁺.

anal: Found: C, 44.04; H, 5.26; N, 9.93 %

Calc. for $\text{C}_{10}\text{H}_{14}\text{N}_2\text{O}_7$: C, 43.80; H, 5.11; N, 10.22 %

(148) N,N'-Oxalo bis(Tyrosine) Dimethyl Ester (MeO-Tyr-CO-CO-Tyr-OMe, 131):

MeO-Tyr-CO-CO-Tyr-OMe (131): (88%)

mp. : crystals from methanol, 232-233°C

ir : ν_{max} (KBr) cm^{-1} : 3283 (NH), 1738 (ester), 1660 (amide I), 1520 (amide II), 1440.

nmr : δ [60 MHz, CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 3.03 (4H, d, $J=6.0$ Hz, Tyr $\text{C}^\beta\text{H}_2 \times 2$), 3.70 (6H, s, $\text{COOCH}_3 \times 2$), 4.70 (2H, m, Tyr $\text{C}^\alpha\text{H} \times 2$), 6.80 (8H, m, aromatic protons), 7.86 (2H, d, $J=7.5$ Hz, exchangeable, Tyr NH $\times 2$).

ms : m/z : 445 (MH)⁺.

anal: Found: C, 59.63; H, 5.38; N, 6.64 %

Calc. for $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8$: C, 59.46; H, 5.41; N, 6.31 %

$[\alpha]_D^{24}$: +24.61 (c, 0.52, MeOH).

(149) N,N'-Oxalo bis(Tryptophan) Dimethyl Ester (MeO-Trp-CO-CO-Trp-OMe, 132):

MeO-Trp-CO-CO-Trp-OMe (132): (60%)

mp. : white flakes from methanol, 177-178°C

ir : ν_{max} (KBr) cm^{-1} : 3410 (NH), 3298 (NH), 1740 (ester), 1661 (amide I), 1518 (amide II), 1457, 1438.

nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 3.34 (4H, d, $J=5.0$ Hz, Trp $\text{C}^\beta\text{H}_2 \times 2$), 3.71 (6H, s, $\text{COOCH}_3 \times 2$), 4.78 (2H, m, Trp $\text{C}^\alpha\text{H} \times 2$), 6.84-7.65 (10H, m, aromatic protons), 8.15 (2H, d, $J=7.5$ Hz.

exchangeable, Trp NHx2), 10.34 (2H, s, exchangeable, Indole NHx2).

ms : m/z: 491 (MH)⁺, 245 (M/2)⁺.

anal: Found: C, 63.73; H, 5.21; N, 11.45 %

Calc. for C₂₆H₂₆N₄O₆: C, 63.67; H, 5.31; N, 11.43 %

[α]_D²⁴: +8.9 (c, 0.55, MeOH).

(150) N,N'-Oxalo bis(Proline) Dimethyl Ester (MeO-Pro-CO-CO-Pro-OMe, 133):

MeO-Pro-CO-CO-Pro-OMe (133): (70%)

mp. : crystals from ethyl acetate, 148-9°C (lit.¹⁷² mp. 154-155°C)

ir : ν_{max} (KBr)cm⁻¹: 1740 (ester), 1662 (amide I), 1639 (amide I), 1541 (amide II).

nmr : δ (CDCl₃): 2.07 (8H, m, Pro C^βH₂x2 + Pro C^γH₂x2), 3.81 (10H, s + m, Pro C^δH₂x2 + COOCH₃x2), 4.53, 5.87 (2H, m, m, Pro C^αHx2).

ms : m/z: 313 (MH)⁺.

anal: Found: C, 53.83; H, 6.94; N, 8.62 %

Calc. for C₁₄H₂₀N₂O₆: C, 53.85; H, 6.41; N, 8.97 %

[α]_D²⁴: -81.68 (c, 1.66, MeOH).

(151) N,N'-Oxalo bis(N^α-Benzyloxycarbonyl Lysine) Methyl Ester

(MeO-Lys(N^αZ)-CO-CO-Lys(N^αZ)-OMe, 134):

(i) N^α-Benzyloxycarbonyl lysine methyl ester hydrochloride

(a) N^ω-Benzylidene lysine:

To an ice cooled and stirred solution of lysine monohydrochloride (9.1g, 50 mmol) in 2N LiOH (25 mL) was added, in drops, benzaldehyde (5.3 mL, 52 mmol). The reaction mixture was kept at 0°C for 5h, filtered, washed with cold water (3x10 mL), methanol (2x10 mL), ether (2x20 mL) and dried in vacuo to give 6.78g (58%) of N^ω-benzylidene lysine.

mp. : 204-205°C (lit.¹⁶⁹ mp. 205-207°C)

nmr : δ (D₂O): 1.09-2.00 (6H, brm, Lys C^βH₂ + C^γH₂ + C^δH₂), 3.15

(2H, m, Lys C^ωH₂), 4.15 (m, 1H, Lys C^αH), 7.20-8.35 (5H, m, aromatic protons), 9.85 (1H, s, imine proton).

(b) N^ωZ-Lys and N^αZ-Lys:

To an ice-cooled and stirred solution of N^ω-benzylidene lysine (11.7g, 50 mmol) in 1N NaOH (50 mL) was added simultaneously, benzyloxycarbonyl chloride (95%) (8.5 mL, 59.75 mmol) and 1N NaOH (75 mL), so that the medium was kept alkaline throughout the experiment. The reaction mixture was left stirred for 0.5h, extracted with ether (2x50 mL), the aqueous layer treated with concentrated HCl (12.5 mL), warmed on a water bath at 50°C for 5 minutes, extracted with ether (2x50 mL), the aqueous layer adjusted to pH 6.2 with 5N NaOH and left aside at 0°C overnight. The precipitated solid was filtered, washed with cold MeOH:EtOAc :: 3:1 (20 mL) and dried to give 2.59g of N^ωZ-Lys.

Yield: 21%

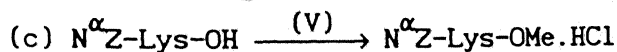
mp. : 251-252°C (lit.¹⁶⁹ mp. 255°C)

The filtrate after removal of N^ωZ-Lys was concentrated to ~50 mL and left aside at 0°C for 5h. The precipitated solid was filtered, washed with cold MeOH:EtOAc::3:1 (10 mL) and dried to give 6.72g (48%) of N^αZ-Lys.

mp. : 232-233°C (lit.¹⁶⁹ mp. 232-233°C)

ir : ν_{\max} (KBr) cm⁻¹: 3320, 1720, 1615, 1520.

nmr : δ (D₂O): 1.00-1.87 (6H, m, Lys C^βH₂ + C^γH₂ + C^δH₂), 3.15 (2H, m, Lys C^ωH₂), 3.89 (1H, m, Lys C^αH), 5.04 (2H, s, Z CH₂), 7.39 (5H, s, aromatic protons).



N^αZ-Lys-OMe.HCl : (54%)

mp. : sticky solid (lit.¹⁶⁹ mp. gummy)

(ii) MeO-Lys(N^αZ)-CO-CO-Lys(N^αZ)-OMe (134): (44%)

mp. : 124-125°C

ir : ν_{\max} (KBr) cm^{-1} : 3290 (NH), 1735 (ester), 1652 (amide I), 1512 (amide II).

nmr : δ (60 MHz, CDCl_3): 1.46 (12H, m, Lys $\text{C}^\beta\text{H}_2 \times 2$ + Lys $\text{C}^\gamma\text{H}_2 \times 2$ + Lys $\text{C}^\delta\text{H}_2 \times 2$), 3.23 (4H, m, Lys $\text{C}^\omega\text{H}_2 \times 2$), 3.70 (6H, s, $\text{COOCH}_3 \times 2$), 4.36 (2H, m, Lys $\text{C}^\alpha\text{H} \times 2$), 5.03 (4H, s, Z $\text{CH}_2 \times 2$), 5.53 (2H, d, $J=7.5$ Hz, exchangeable, Lys $\text{N}^\omega\text{H} \times 2$), 7.23 (10H, s, aromatic protons), 7.60 (2H, m, exchangeable, $\text{N}^\alpha\text{H} \times 2$).

ms : m/z : 643 (MH)⁺.

anal: Found: C, 59.49; H, 6.28; N, 8.29 %

Calc. for $\text{C}_{32}\text{H}_{42}\text{N}_4\text{O}_{10}$: C, 59.81; H, 6.54; N, 8.72 %

(152) N,N'-Oxalo bis(α -Aminoisobutyric Acid) Dimethyl Ester

(MeO-Aib-CO-CO-Aib-OMe, 135):

MeO-Aib-CO-CO-Aib-OMe (135): (70%)

mp. : crystals from methanol, 167-168°C

ir : ν_{\max} (KBr) cm^{-1} : 3297 (NH), 1729 (ester), 1671 (amide I), 1504 (amide II).

nmr : δ [400 MHz, $(\text{CD}_3)_2\text{SO}$]: 1.41 (12H, s, Aib $\text{CH}_3 \times 4$), 3.62 (6H, s, $\text{COOCH}_3 \times 2$), 8.90 (2H, s, exchangeable, Aib $\text{NH} \times 2$).

ms : m/z : 289 (MH)⁺.

anal: Found: C, 50.29; H, 6.83; N, 10.18 %

Calc. for $\text{C}_{12}\text{H}_{20}\text{N}_2\text{O}_6$: C, 50.00; H, 6.94; N, 9.72 %

(153) N,N'-Oxalo bis(L-Alanyl L-Alanine) Dimethyl Ester

(MeO-Ala-Ala-CO-CO-Ala-Ala-OMe, 136):

(i) MeO-Ala-CO-CO-Ala-OMe (125) $\xrightarrow{\text{Method IX}}$ HO-Ala-CO-CO-Ala-OH (146)

HO-Ala-CO-CO-Ala-OH (146): (77%)

mp. : 194-195°C (lit.¹⁷³ mp. 195-205°C)

(ii) HO-Ala-CO-CO-Ala-OH + 2 H-Ala-OMe.HCl

$\xrightarrow{\text{Method VI}}$ MeO-Ala-Ala-CO-CO-Ala-Ala-OMe (136)

MeO-Ala-Ala-CO-CO-Ala-Ala-OMe (136): (63%)

mp. : 216-217°C

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3318 (NH), 3271 (NH), 1737 (ester), 1641 (amide I), 1521 (amide II), 1506.

nmr : δ [400 MHz, $(\text{CD}_3)_2\text{SO}$]: 1.26 (12H, m, Ala $\text{CH}_3 \times 4$), 3.72 (6H, s, $\text{COOCH}_3 \times 2$), 4.26 (4H, m, Ala $\text{C}^\alpha\text{H} \times 4$), 8.48 (4H, m, exchangeable, Ala $\text{NH} \times 4$).

ms : m/z: 403 $(\text{MH})^+$, 201 $(\text{M}/2)^+$.

anal: Found: C, 48.13; H, 6.74; N, 13.83 %

Calc. for $\text{C}_{16}\text{H}_{26}\text{N}_4\text{O}_8$: C, 47.76; H, 6.47; N, 13.93 %

$[\alpha]_D^{24}$: -56.36 (c, 0.33, MeOH).

(154) N,N'-Oxalo bis(L-Leucyl L-Leucine) Dimethyl Ester

(MeO-Leu-Leu-CO-CO-Leu-Leu-OMe, 137):

(i) MeO-Leu-CO-CO-Leu-OMe (127) $\xrightarrow{\text{Method IX}}$ HO-Leu-CO-CO-Leu-OH (148)

HO-Leu-CO-CO-Leu-OH (148): (93%)

mp. : 160-161°C

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3274, 1725, 1678, 1514, 1469.

ms : m/z: 317 $(\text{MH})^+$.

(ii) HO-Leu-CO-CO-Leu-OH + 2 H-Leu-OMe.HCl

$\xrightarrow{\text{Method VI}}$ MeO-Leu-Leu-CO-CO-Leu-Leu-OMe (137)

MeO-Leu-Leu-CO-CO-Leu-Leu-OMe (137): (65%)

mp. : 200-201°C

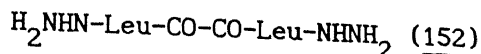
ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3261 (NH), 1747 (ester), 1651 (amide I), 1536 (amide II), 1510.

nmr : δ (CDCl_3): 0.90 (24H, brs, Leu $\text{CH}_3 \times 8$), 1.62 (12H, m, Leu $\text{C}^\beta\text{H}_2 \times 4$ + Leu $\text{C}^\gamma\text{H} \times 4$), 3.75 (6H, s, $\text{COOCH}_3 \times 2$), 4.50 (4H, m, Leu $\text{C}^\alpha\text{H} \times 4$), 6.59 (2H, d, $J=7.5$ Hz, exchangeable, $\text{NH} \times 2$), 7.93 (2H, d, $J=7.5$ Hz, exchangeable, $\text{NH} \times 2$).

ms : m/z: 571 $(\text{MH})^+$, 285 $(\text{M}/2)^+$.

anal: Found: C, 58.83; H, 8.68; N, 9.73 %

(155) N,N'-Oxalo bis(L-Leucyl L-Alanine) Dimethyl Ester

(MeO-Ala-Leu-CO-CO-Leu-Ala-OMe, 138):(i) MeO-Leu-CO-CO-Leu-OMe (127) $\xrightarrow{\text{Method XII}}$ 

$$\text{H}_2\text{NHN-Leu-CO-CO-Leu-NHNH}_2 \text{ (152): (93%)}$$

mp. : 219-220°C

(ii) $\text{H}_2\text{NHN-Leu-CO-CO-Leu-NHNH}_2 + 2 \text{ H-Ala-OMe.HCl}$

$$\xrightarrow{\text{Method VII}} \text{MeO-Ala-Leu-CO-CO-Leu-Ala-OMe (138)}$$
MeO-Ala-Leu-CO-CO-Leu-Ala-OMe (138): (65%)

mp. : 184-185°C

ir : ν_{max} (KBr) cm^{-1} : 3285 (NH), 1738 (ester), 1635 (amide I),
1505 (amide II).

(156) N,N'-Oxalo bis(L-Leucyl L-Serine) Dimethyl Ester

(MeO-Ser-Leu-CO-CO-Leu-Ser-OMe, 139):(i) MeO-Leu-CO-CO-Leu-OMe (127) $\xrightarrow{\text{Method IX}}$ HO-Leu-CO-CO-Leu-OH (148)(ii) HO-Leu-CO-CO-Leu-OH (148) + 2 H-Ser-OMe.HCl
$$\xrightarrow{\text{Method VI}} \text{MeO-Ser-Leu-CO-CO-Leu-Ser-OMe (139)}$$
MeO-Ser-Leu-CO-CO-Leu-Ser-OMe (139): (55%)

mp. : 205-206°C

ir : ν_{max} (KBr) cm^{-1} : 3331 (NH), 1766, 1738 (ester), 1659 (amide
I), 1523 (amide II).

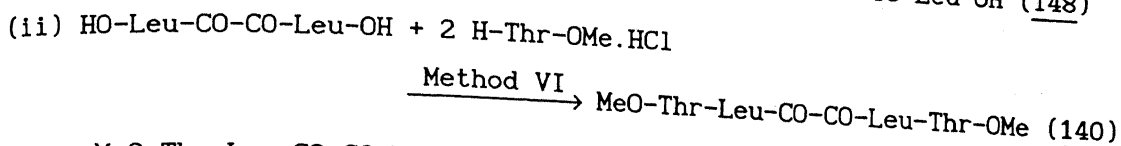
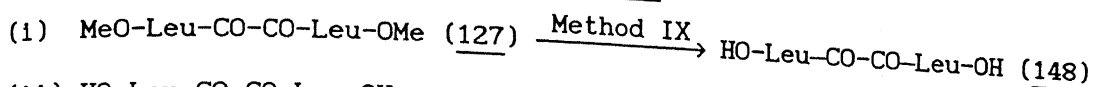
nmr : δ (CDCl_3): 0.90 (12H, brs, Leu $\text{CH}_3 \times 4$), 1.75 (6H, m, Leu
 $\text{C}^\beta\text{H}_2 \times 2 + \text{Leu } \text{C}^\gamma\text{H} \times 2$), 3.78 (10H, s + m, $\text{COOCH}_3 \times 2 + \text{Ser}$
 $\text{C}^\beta\text{H}_2 \times 2$), 4.62 (2H, m, Ser $\text{C}^\alpha\text{H} \times 2$), 5.00 (2H, m, Leu $\text{C}^\alpha\text{H} \times 2$),
8.43 (4H, br, exchangeable, Ser $\text{NH} \times 2 + \text{Leu } \text{NH} \times 2$).

ms : m/z: 519 (MH)⁺, 259 (M/2)⁺.

anal: Found: C, 50.86; H, 7.38; N, 10.49 %

Calc. for $\text{C}_{22}\text{H}_{38}\text{N}_4\text{O}_{10}$: C, 50.96; H, 7.33; N, 10.81 % $[\alpha]_D^{24}$: -32.85 (c, 0.70, MeOH).

(157) N,N'-Oxalo bis(L-Leucyl L-Threonine) Dimethyl Ester

(MeO-Thr-Leu-CO-CO-Leu-Thr-OMe, 140):MeO-Thr-Leu-CO-CO-Leu-Thr-OMe (140): (80%)

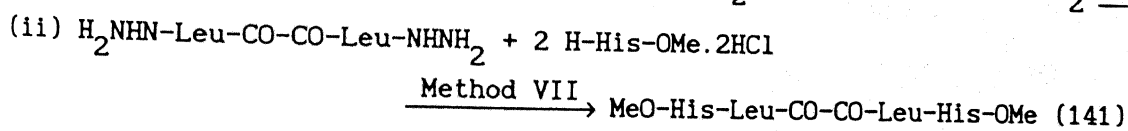
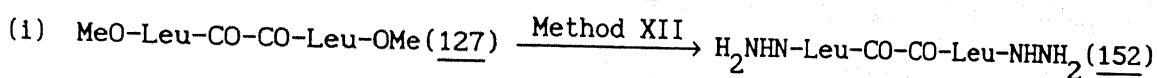
mp. : 197-198°C

ir : ν_{max} (KBr) cm^{-1} : 3326 (NH), 1741 (ester), 1654 (amide I), 1626 (amide I), 1575 (amide II), 1522 (amide II).nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 0.96 (12H, d, $J=5.0$ Hz, Leu $\text{CH}_3 \times 4$), 1.12 (6H, d, $J=6.0$ Hz, Thr $\text{CH}_3 \times 2$), 1.68 (6H, m, Leu $\text{C}^\beta\text{H}_2 \times 2$ + Leu $\text{C}^\gamma\text{H} \times 2$), 3.71 (6H, s, $\text{COOCH}_3 \times 2$), 4.14-4.80 (6H, m, Thr $\text{C}^\alpha\text{H} \times 2$ + Thr $\text{C}^\beta\text{H} \times 2$ + Leu $\text{C}^\alpha\text{H} \times 2$), 5.46 (2H, d, $J=7.5$ Hz, exchangeable, Thr $\text{OH} \times 2$), 8.06 (2H, d, $J=7.5$ Hz, exchangeable, $\text{NH} \times 2$), 8.56 (2H, d, $J=7.5$ Hz, exchangeable, $\text{NH} \times 2$).ms : m/z : 547 (MH^+), 273 ($\text{M}/2^+$).

anal: Found: C, 52.39; H, 7.36; N, 10.69 %

Calc. for $\text{C}_{24}\text{H}_{42}\text{N}_4\text{O}_{10}$: C, 52.75; H, 7.69; N, 10.26 % $[\alpha]_D^{24}$: -21.90 (c, 0.63, MeOH).

(158) N,N'-Oxalo bis(L-Leucyl L-Histidine) Dimethyl Ester

(MeO-His-Leu-CO-CO-Leu-His-OMe, 141):MeO-His-Leu-CO-CO-Leu-His-OMe (141): (43%)

mp. : 124-126°C

ir : ν_{max} (KBr) cm^{-1} : 3342 (NH), 1737 (ester), 1668 (amide I), 1547 (amide II), 1512 (amide II).nmr : δ [400 MHz, CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 0.92 (12H, m, Leu $\text{CH}_3 \times 4$),

1.66 (6H, m, Leu C^βH₂x2 + Leu C^γHx2), 3.10 (4H, m, His C^βH₂x2), 3.74 (6H, s, COOCH₃x2), 4.52 (2H, m, Leu C^αHx2), 4.75 (2H, m, His C^αHx2), 6.78 (2H, s, Imidazole ⁴Hx2), 7.50 (2H, s, Imidazole ²Hx2), 8.16 (2H, d, J=7.5 Hz, NHx2), 8.26 (2H, d, J=7.5 Hz, NHx2).

ms : m/z: 619 (MH)⁺, 309 (M/2)⁺.

anal: Found: C, 54.37; H, 6.48; N, 18.22 %

Calc. for C₂₈H₄₂N₈O₈: C, 54.37; H, 6.80; N, 18.12 %

[α]_D²⁴: -34.76 (c, 0.86, MeOH).

(159) N,N'-Oxalo bis(L-Leucyl L-Leucyl L-Leucine) Dimethyl Ester

(MeO-Leu-Leu-Leu-CO-CO-Leu-Leu-Leu-OMe, 142):

(i) MeO-Leu-Leu-CO-CO-Leu-Leu-OMe (137) Method IX,

HO-Leu-Leu-CO-CO-Leu-Leu-OH (153)

HO-Leu-Leu-CO-CO-Leu-Leu-OH (153): (63%)

mp. : 153-154°C

(ii) HO-Leu-Leu-CO-CO-Leu-Leu-OH + 2 H-Leu-OMe.HCl

Method VI → MeO-Leu-Leu-Leu-CO-CO-Leu-Leu-Leu-OMe (142)

MeO-Leu-Leu-Leu-CO-CO-Leu-Leu-Leu-OMe (142): (76%)

mp. : 283-284°C

ir : ν_{max} (KBr)cm⁻¹: 3280 (NH), 1730 (ester), 1635 (amide I), 1528 (amide II), 1490.

nmr : δ (CDCl₃): 0.84 (36H, brs, Leu CH₃x12), 1.62 (18H, m, Leu C^βH₂x6 + Leu C^γHx6), 3.68 (6H, s, COOCH₃x2), 4.40 (3H, m, Leu C^αHx3), 4.90 (3H, m, Leu C^αHx3), 8.00-9.71 (6H, br, NHx6).

ms : m/z: 797 (MH)⁺.

anal: Found: C, 60.21; H, 9.18; N, 10.43 %

Calc. for C₄₀H₇₂N₆O₁₀: C, 60.30; H, 9.04; N, 10.55 %

[α]_D²⁴: -108.0 (c, 0.43, CHCl₃).

(160) N,N'-Oxalo bis(L-Leucyl L-Leucyl L-Alanine) Dimethyl Ester

(MeO-Ala-Leu-Leu-CO-CO-Leu-Leu-Ala-OMe, 143):(i) MeO-Leu-Leu-CO-CO-Leu-Leu-OMe (137) Method IX,HO-Leu-Leu-CO-CO-Leu-Leu-OH (153)

(ii) HO-Leu-Leu-CO-CO-Leu-Leu-OH + 2 H-Ala-OMe.HCl

Method VI → MeO-Ala-Leu-Leu-CO-CO-Leu-Leu-Ala-OMe (143)MeO-Ala-Leu-Leu-CO-CO-Leu-Leu-Ala-OMe (143): (72%)

mp. : 156-157°C

ir : ν_{\max} (KBr) cm^{-1} : 3298 (NH), 3072, 1747 (ester), 1650 (amide I), 1546 (amide II), 1453.nmr : δ (CDCl_3): 0.87 (24H, brs, Leu $\text{CH}_3 \times 8$), 1.31 (6H, d, $J=6.5$ Hz, Ala $\text{CH}_3 \times 2$), 1.62 (12H, m, Leu $\text{C}^\beta \text{H}_2 \times 4$ + Leu $\text{C}^\gamma \text{H} \times 4$), 3.75 (6H, s, $\text{COOCH}_3 \times 2$), 4.53 (6H, m, Ala $\text{C}^\alpha \text{H} \times 2$ + Leu $\text{C}^\alpha \text{H} \times 4$), 7.43 (4H, m, $\text{NH} \times 4$), 8.31 (2H, m, $\text{NH} \times 2$).

anal: Found: C, 57.36; H, 8.16; N, 11.64 %

Calc. for $\text{C}_{34}\text{H}_{60}\text{N}_6\text{O}_{10}$: C, 57.30; H, 8.43; N, 11.80 % $[\alpha]_D^{24}$: -46.93 (c, 0.75, MeOH).

(161) N,N'-Oxalo bis(L-Leucyl L-Alanyl Glycine) Dimethyl Ester

(MeO-Gly-Ala-Leu-CO-CO-Leu-Ala-Gly-OMe, 144):(i) MeO-Ala-Leu-CO-CO-Leu-Ala-OMe (138)Method XII → $(\text{CO-Leu-Ala-NHNH}_2)_2$ (154) $\text{H}_2\text{NHN-Ala-Leu-CO-CO-Leu-Ala-NHNH}_2$ (154): (78%)

mp. : 223-234°C

(ii) $\text{H}_2\text{NHN-Ala-Leu-CO-CO-Leu-Ala-NHNH}_2$ + 2 H-Gly-OMe.HClMethod VII → MeO-Gly-Ala-Leu-CO-CO-Leu-Ala-Gly-OMe (144)MeO-Gly-Ala-Leu-CO-CO-Leu-Ala-Gly-OMe (144): (30%)

mp. : crystals from methanol, 235-236°C

ir : ν_{\max} (KBr) cm^{-1} : 3280 (NH), 3045, 1739 (ester), 1628 (amide I), 1520 (amide II), 1500.

nmr : δ [400 MHz, $\text{CDCl}_3 + 2\% (\text{CD}_3)_2\text{SO}$]: 0.95 (6H, d, $J=5.0$ Hz, Leu $\text{CH}_3 \times 2$), 1.00 (6H, d, $J=5.0$ Hz, Leu $\text{CH}_3 \times 2$), 1.31 (6H, d, $J=6.5$ Hz, Ala $\text{CH}_3 \times 2$), 1.70 (6H, m, Leu $\text{C}^\beta\text{H}_2 \times 2 + \text{Leu C}^\gamma\text{H} \times 2$), 3.75 (6H, s, $\text{COOCH}_3 \times 2$), 3.87 (2H, dd, Gly $\text{CH} \times 2$), 4.20 (2H, dd, Gly $\text{CH} \times 2$), 4.37 (2H, m, Leu $\text{C}^\alpha\text{H} \times 2$), 4.58 (2H, m, Ala $\text{C}^\alpha\text{H} \times 2$), 7.34 (2H, br, Gly $\text{NH} \times 2$), 7.47 (2H, d, $J=7.5$ Hz, Ala $\text{NH} \times 2$), 7.96 (2H, d, $J=7.5$ Hz, Leu $\text{NH} \times 2$).

^{13}C nmr : δ [100.57 MHz, $\text{CDCl}_3 + (\text{CD}_3)_2\text{SO}$]: 17.42 (Leu CH_3), 21.36 (Ala CH_3), 22.75 (Leu C^βH_2), 24.58 (Leu C^γH), 40.80 (Gly CH_2), 48.32 (COOCH_3), 52.03 (Leu C^αH), 170.53, 171.04 (Leu CO, Ala CO), 172.45 (Gly CO).

ms : m/z : 600 (M) $^+$.

anal: Found: C, 52.56; H, 6.87; N, 14.32 %

Calc. for $\text{C}_{26}\text{H}_{44}\text{N}_6\text{O}_{10}$: C, 52.00; H, 7.33; N, 14.00 %

(162) (Gly-CO-CO-Gly) Cu_2 (156):

(i) $\text{MeO-Gly-CO-CO-Gly-OMe}$ (124) $\xrightarrow{\text{Method IX}}$ $\text{HO-Gly-CO-CO-Gly-OH}$ (145)

$\text{HO-Gly-CO-CO-Gly-OH}$ (145): (62%)

mp. : 247-248 $^\circ\text{C}$ (lit. 173 mp. 250 $^\circ\text{C}$)

(ii) (Gly-CO-CO-Gly) Cu_2 (156):

$\text{HO-Gly-CO-CO-Gly-OH}$ (145, 0.204g, 1 mmol) was dissolved in aq. NaHCO_3 (0.252g, 3 mmol in 5 mL water), admixed with a clear solution of $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ (0.482 g, 2 mmol) in 5 mL water, resulting in a greenish blue solution. The solution on slow evaporation at room temperature yielded sky blue powdery solid, 0.23 g (70%).

mp. : colour changes from blue to green at 260-270 $^\circ\text{C}$, changes to black at 320-325 $^\circ\text{C}$, does not melt.

ir : ν_{max} (KBr) cm^{-1} : 3200 (br, NH), 1620 (br), 1375, 1300, 1280.

ms : m/z : 329 (M) $^+$.

(163) (Aib-CO-CO-Aib) Cu_2 (157):

(i) $\text{MeO-Aib-CO-CO-Aib-OMe}$ (135) $\xrightarrow{\text{Method IX}}$ $\text{HO-Aib-CO-CO-Aib-OH}$ (147)

HO-Aib-CO-CO-Aib-OH (147): (79%)

mp. : 280-281°C (lit.¹⁷⁴ mp. 284°C)

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3297 (NH), 1740, 1656 (amide I), 1522 (amide II), 1470.

ms : m/z: 261 (MH)⁺.

(ii) (Aib-CO-CO-Aib)Cu₂ (157):

HO-Aib-CO-CO-Aib-OH (0.260 g, 1 mmol), was dissolved in aq. NaHCO₃ (0.210g, 2.5 mmol in 5 mL water), filtered to get a clear solution and added to a clear solution of Cu(NO₃)₂·3H₂O (0.483 g, 2 mmol in minimum amount of water). Immediately on mixing the solution, the colour changes to turquoise blue and after ~10-15 min. of standing at room temperature crystals were formed which were filtered, washed with chilled water and air dried, 0.338 g, (95%).

The turquoise blue coloured crystals, on drying in vacuo, turned deep blue. The turquoise colour was restored when dried crystals were redissolved in hot water.

mp. : colour changes from blue to dark green at 260-270°C, turns black at 320-325°C, does not melt upto 350°C.

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3363, 1712, 1644, 1562, 1500, 1466, 1421, 1325, 1182.

ms : m/z: 321 (M - Cu)⁺.

(164) (Leu-CO-CO-Leu)Cu₂ (158):

(i) MeO-Leu-CO-CO-Leu-OMe (127) $\xrightarrow{\text{Method IX}}$ HO-Leu-CO-CO-Leu-OH (148)

HO-Leu-CO-CO-Leu-OH (148): [experiment No. 155]

(ii) (Leu-CO-CO-Leu)Cu₂ (158):

HO-Leu-CO-CO-Leu-OH (155, 0.314g, 1 mmol) was dissolved in methanol (~10 mL), admixed with 10 mL water and basic cupric carbonate (0.96 g, 4 mmol), heated to boiling for ~10 min. using a bunsen burner and filtered hot through a sintered funnel. The filtrate on slow evaporation at room temperature gave light blue

mp. : colour changes to black at 260-270°C, does not melt upto 310°C.

ir : ν_{\max} (KBr) cm^{-1} : 3385, 3302 (br), 1665, 1512, 1468.

(165) (Tyr-CO-CO-Tyr)Cu₂ (159):

(i) MeO-Tyr-CO-CO-Tyr-OMe (131) $\xrightarrow{\text{Method IX}}$ HO-Tyr-CO-CO-Tyr-OH (149)
HO-Tyr-CO-CO-Tyr-OH (149): (87%)

mp. : 244-245°C (lit.¹⁷³ mp. 245-247°C)

(ii) (Tyr-CO-CO-Tyr)Cu₂ (159):

Tyr-CO-CO-Tyr (0.208g, 0.5 mmol) was dissolved in methanol (~10 mL). admixed with 10 mL water and basic cupric carbonate (0.48 g, 2 mmol), heated to boiling for ~10 min. using a bunsen burner and filtered hot through a sintered funnel. The dark bluish green filtrate on slow evaporation at room temperature gave tiny bottle green shining crystals, recrystallized from MeOH, 0.31 g (91%).

mp. : colour changes to black at 245-250°C, does not melt upto 310°C.

ir : ν_{\max} (KBr) cm^{-1} : 3382 (br), 1658, 1588, 1544, 1513, 1442, 1422, 1364, 1314, 1277.

ms : m/z: 541 (M)⁺.

(166) (Trp-CO-CO-Trp)Cu₂ (160):

(i) MeO-Trp-CO-CO-Trp-OMe (132) $\xrightarrow{\text{Method IX}}$ HO-Trp-CO-CO-Trp-OH (150)
HO-Trp-CO-CO-Trp-OH (150): (80%)

mp. : 215-217°C

ir : ν_{\max} (KBr) cm^{-1} : 3403 (NH), 3313 (NH), 1736 (acid), 1664 (amide I), 1527 (amide II), 1455, 1424.

ms : m/z: 463 (MH)⁺.

(ii) (Trp-CO-CO-Trp)Cu₂ (160):

HO-Trp-CO-CO-Trp-OH (157, 0.462g, 1 mmol) was dissolved in

methanol (~10 mL), admixed with 10 mL of water and basic cupric carbonate (0.96 g, 4 mmol), heated to boiling for ~10 min. using a bunsen burner and filtered hot through a sintered funnel. The deep green filtrate on slow evaporation at room temperature gave dark green shining flakes, recrystallized from hot methanol, 0.575 g (98%).

mp. : starts turning black at 190°C, becomes totally black at 200°C and decomposes at 255-260°C.

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3397 (br), 1654 (br), 1506, 1456, 1418, 1341, 743.

ms : m/z: 587 (M)⁺.

(167) (Pro-CO-CO-Pro)Cu₂ (161):

(i) MeO-Pro-CO-CO-Pro-OMe (133) $\xrightarrow{\text{Method IX}}$ HO-Pro-CO-CO-Pro-OH (151)
HO-Pro-CO-CO-Pro-OH (151): (76%)

mp. : 79-80°C

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 2983 (br), 2591 (br), 1741 (acid), 1638 (br, amide I), 1513 (amide II), 1456, 1408, 1316.

(ii) (Pro-CO-CO-Pro)Cu₂ (161):

HO-Pro-CO-CO-Pro-OH (151, 0.284g, 1 mmol) was dissolved in methanol (~20 mL), admixed with 10 mL of water and basic cupric carbonate (0.96 g, 4 mmol), refluxed for 2h, and filtered hot through a sintered funnel. The filtrate on slow evaporation at room temperature yielded turquoise blue hygroscopic solid, turns dark blue on leaving outside, 0.37 g (90%).

mp. : colour changes to black at 205-208°C and melts at 232-204°C.

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 2925 (br), 1741, 1617, 1513, 1455, 1407, 1346, 1215, 1184.

ms : m/z: 444 (M⁺ + 2H₂O - 1)⁺.

(168) $(N^{\alpha}Z\text{-Lys-CO-CO-Lys-}N^{\alpha}Z)Cu_2$ (162):

(i) $MeO\text{-}(N^{\alpha}Z)\text{Lys-CO-CO-Lys}(N^{\alpha}Z)\text{-OMe}$ (134)

Method IX \rightarrow $HO\text{-}(N^{\alpha}Z)\text{Lys-CO-CO-Lys}(N^{\alpha}Z)\text{-OH}$ (155)

$HO\text{-}(N^{\alpha}Z)\text{Lys-CO-CO-Lys}(N^{\alpha}Z)\text{-OH}$ (162): (73%)

mp. : sticky solid

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3330 (br, NH), 2940 (br), 1685 (br, amide I), 1508 (br, amide II).

(ii) $(N^{\alpha}Z\text{-Lys-CO-CO-Lys-}N^{\alpha}Z)Cu_2$ (162):

$HO\text{-}(N^{\alpha}Z)\text{Lys-CO-CO-Lys}(N^{\alpha}Z)\text{-OH}$ (155, 0.614 g, 1 mmol) was dissolved in MeOH (~10 mL), admixed with water (~10 mL) and basic cupric carbonate (0.96 g, 4 mmol), refluxed for 2h, in an oil bath and filtered hot through a sintered funnel. The blue filtrate on slow evaporation at room temperature yielded shining, blue needles, 0.37 g, (50%).

mp. : turns black at 250-260°C, does not melt upto 335°C.

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3540, 3450, 1670 (br), 1410, 1370, 800.

ms : m/z: 740 $(M + 1)^+$.

A typical procedure for the generation of Δ Ala residue from a serine residue, in peptides, is as follows:

A solution of oxalyl chloride (0.14 mL, 1.5 mmol) in dry CH_2Cl_2 (2 mL) was added dropwise to a well stirred solution of Ser-peptide (1 mmol) in dry CH_2Cl_2 (10 mL) [or where insoluble, in THF/EtOAc] containing triethylamine (0.42 mL, 3 mmol) at 0°C over ~ 0.25 h. The reaction was monitored by the disappearance of the starting material (tlc, average time 2-4 h at room temperature). The reaction mixture was washed with 5% NaHCO_3 , dried (anhyd. MgSO_4), evaporated in vacuo and the residue chromatographed on a short column of silica gel. Elution with ethyl acetate - benzene afforded pure dehydropeptide.

(169) Reaction of Bz-Ser-OMe (1) with $(\text{COCl})_2$: Isolation of N-Benzoyl Δ Alanine Methyl Ester (Bz- Δ Ala-OMe 163):

(i) Bz-Ser-OMe (1): experiment No. 1.

(ii) Bz- Δ Ala-OMe (163): (90%)

mp. : syrup

ir : ν_{max} (neat) cm^{-1} : 3323 (NH), 1776 ($\text{CONHC}=\text{CH}_2$), 1743 (ester), 1673 (br, amide I), 1530 (amide II), 1454.

nmr : δ [CDCl_3 + $(\text{CD}_3)_2\text{SO}$]: 3.84 (3H, s, COOCH_3), 5.87, 6.50 (1H, 1H, s, s, $=\text{CH}_2$), 7.1-8.1 (5H, m, aromatic protons), 8.93 (1H, br, Δ Ala NH).

anal: Found: C, 64.44; H, 5.27; N, 6.83 %

Calc. for $\text{C}_{11}\text{H}_{11}\text{NO}_3$: C, 64.39; H, 5.37; N, 6.83 %

(170) Reaction of Z-Gly-Ser-OMe (5) with $(\text{COCl})_2$: Isolation of N-Benzoyloxycarbonyl-Glycyl- Δ Alanine Methyl Ester (Z-Gly- Δ Ala-OMe, 164):

(i) Z-Gly-Ser-OMe (5): experiment No. 3.

(ii) Z-Gly- Δ Ala-OMe (164): (40%)

mp. : syrup

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3360 (NH), 1720 (br, ester), 1680 (amide I), 1510 (br, amide II), 1435.

nmr : δ (CDCl_3): 3.84 (3H, s, COOCH_3), 3.71-4.0 (2H, m, Gly CH_2), 5.15 (2H, s, Z CH_2), 5.59 (1H, m, Gly NH), 5.90, 6.59 (1H, 1H, s, s, $=\text{CH}_2$), 7.34 (5H, s, aromatic protons), 8.25 (1H, brs, Δ Ala NH).

ms : m/z: 293 (MH)⁺.

anal: Found: C, 57.43; H, 5.80; N, 9.36 %

Calc. for $\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}_5$: C, 57.53; H, 5.48; N, 9.59 %

(171) Reaction of Bz-Ala-Ser-OMe (9) with $(\text{COCl})_2$: Isolation of N-Benzoyl L-Alanyl- Δ Alanine Methyl Ester (Bz-ALA- Δ Ala-OMe, 165):

(i) Bz-Ala-Ser-OMe (9): experiment No. 5.

(ii) Bz-Ala- Δ Ala-OMe (165): (58%)

mp. : 110-115°C

ir : $\nu_{\max}(\text{KBr})\text{cm}^{-1}$: 3310 (NH), 1720 (ester), 1680 (amide I), 1623 (amide I), 1505 (br, amide II).

nmr : δ (60 MHz, CCl_4): 1.45 (3H, d, J=6.5 Hz, Ala CH_3), 3.78 (3H, s, COOCH_3), 4.75 (1H, m, Ala C^αH), 5.81, 6.53 (1H, 1H, s, s, $=\text{CH}_2$), 7.1-8.0 (6H, m, Ala NH + aromatic protons), 8.36 (1H, brs, Δ Ala NH).

ms : m/z: 277 (MH)⁺.

anal: Found: C, 60.49; H, 5.88; N, 10.26 %

Calc. for $\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}_4$: C, 60.87; H, 5.80; N, 10.14 %

$[\alpha]_D^{23}$: -37.18 (c, 0.43, CHCl_3).

(172) Reaction of Bz-Leu-Ser-OMe (11) with $(\text{COCl})_2$: Isolation of N-Benzoyl

L-Leucyl- Δ Alanine Methyl Ester (Bz-Leu- Δ Ala-OMe, 166):

(i) Bz-Leu-Ser-OMe (11): experiment No. 6.

(ii) Bz-Leu- Δ Ala-OMe (166): (56%)

mp. : 55-56°C

ir : ν_{\max} (KBr) cm^{-1} : 3330 (NH), 1720 (ester), 1660 (amide I), 1625 (amide I), 1525 (amide II), 1475.

nmr : δ (60 MHz, CCl_4): 0.96 (6H, d, $J=5.0$ Hz, Leu $\text{CH}_3 \times 2$), 1.70 (3H, m, Leu C^βH_2 + Leu C^γH), 3.73 (3H, s, COOCH_3), 4.70 (1H, m, Leu C^αH), 5.83, 6.53 (1H, 1H, s, s, $=\text{CH}_2$), 7.13-7.90 (6H, m, Leu NH + aromatic protons), 8.43 (1H, brs, exchangeable with D_2O , Δ Ala NH).

ms : m/z : 319 (MH) $^+$.

anal: Found: C, 64.22; H, 6.87; N, 8.58 %

Calc. for $\text{C}_{17}\text{H}_{22}\text{N}_2\text{O}_4$: C, 64.15; H, 6.92; N, 8.81 %

(173) Reaction of Z-Phe-Ser-OMe (167) with $(\text{COCl})_2$: Isolation of N-Benz-yloxycarbonyl L-Phenylalanyl- Δ Alanine Methyl Ester (Z-Phe- Δ Ala-OMe, 168):

(i) N-Benz-yloxycarbonyl L-phenylalanyl-L-serine-methyl ester (Z-Phe-Ser-OMe, 167):

Z-Phe-OH + H-Ser-OMe.HCl $\xrightarrow{\text{Method VI}}$ Z-Phe-Ser-OMe (167)

Z-Phe-Ser-OMe (167): (92%)

mp. : 83-84 $^\circ\text{C}$

ir : ν_{\max} (KBr) cm^{-1} : 3305 (NH), 1735 (ester), 1698 (carbamate), 1642 (amide I), 1530 (amide II).

nmr : δ (CDCl_3): 3.13 (2H, m, Phe C^βH_2), 3.70 (3H, s, COOCH_3), 3.80 (2H, m, Ser C^βH_2), 4.56 (2H, m, Phe C^αH + Ser C^αH), 5.00 (2H, s, Z CH_2), 5.83 (1H, d, $J=7.5$ Hz, Phe NH), 7.03-7.46 (11H, m, Ser NH + aromatic protons).

(ii) Z-Phe- Δ Ala-OMe (168): (58%)

mp. : syrup

ir : ν_{\max} (neat) cm^{-1} : 3060, 1740 (br), 1643, 1500.

nmr : δ (CDCl_3): 3.40 (2H, m, Phe C^βH_2), 3.75 (3H, s, COOCH_3), 4.87 (1H, m, Phe C^αH), 5.41 (2H, s, Z CH_2), 5.43, 6.53 (1H, 1H, s, s, $=\text{CH}_2$), 7.03-7.65 (12H, m, Phe NH + Δ Ala NH +

ms : m/z: 383 (MH)⁺.

anal: Found: C, 65.83; H, 5.58; N, 7.43 %

Calc. for C₂₁H₂₂N₂O₅: C, 65.97; H, 5.76; N, 7.33 %

[α]_D²³: +62.99 (c, 1.77, CHCl₃).

(174) Reaction of Bz-Val-Ser-OMe (27) with (COCl)₂ : Isolation of N-Benzoyl L-Valyl-Δ Alanine Methyl Ester (Bz-Val-Δ Ala-OMe, 169):

(i) Bz-Val-Ser-OMe (27): experiment No. 14.

(ii) Bz-Val-Δ Ala-OMe (169): (48%)

mp. : syrup

ir : ν_{max} (neat)cm⁻¹: 3315, (br, NH), 1820, 1725 (br, ester), 1638 (amide I), 1520 (amide II).

nmr : δ (CDCl₃): 0.75-1.25 (6H, m, Val CH₃×2), 2.18 (1H, m, Val C^βH), 3.78 (3H, s, COOCH₃), 4.53 (1H, m, Val C^αH), 5.84, 6.53 (1H, 1H, s, s, =CH₂), 7.10-7.93 (6H, m, Val NH + aromatic protons), 8.25 (1H, brs, Δ Ala NH).

ms : m/z: 305 (M)⁺.

anal: Found: C, 62.83; H, 6.64; N, 9.11 %

Calc. for C₁₆H₂₁N₂O₄: C, 62.95; H, 6.89; N, 9.18 %

[α]_D²³: +4.90 (c, 1.06, CHCl₃).

(175) Reaction of Bz-Pro-Ser-OMe (29) with (COCl)₂ : Isolation of N-Benzoyl L-Prolyl-Δ Alanine Methyl Ester (170):

(i) Bz-Pro-Ser-OMe (29): experiment No. 15.

(ii) Bz-Pro-Δ Ala-OMe (170): (54%)

mp. : 110-111°C

ir : ν_{max} (KBr)cm⁻¹: 3390 (NH), 1730 (ester), 1685 (amide I), 1612 (amide I), 1510 (amide II).

nmr : δ (CDCl₃): 1.62-2.5 (4H, m, Pro C^βH₂ + Pro C^γH₂), 3.53 (2H, t, Pro C^δH₂), 3.78 (3H, s, COOCH₃), 4.78 (1H, m, Pro C^αH), 5.84, 6.53 (1H, 1H, s, s, =CH₂), 7.21-7.59 (5H, m, aromatic

protons), 8.96 (1H, brs, exchangeable with D_2O , Δ Ala NH).

ms : m/z: 303 (MH)⁺.

anal: Found: C, 63.42; H, 5.83; N, 9.18 %

Calc. for $C_{16}H_{18}N_2O_4$: C, 63.58; H, 5.96; N, 9.27 %

$[\alpha]_D^{23}$: -86.16 (c, 0.73, $CHCl_3$).

(176) Reaction of Z-Pro-Ser-OMe (171) with $(COCl)_2$: Isolation of N-Benzyloxycarbonyl L-Prolyl- Δ Alanine Methyl Ester (Z-Pro- Δ Ala OMe, 172):

(i) N-Benzyloxycarbonyl L-prolyl-L-serine-methyl ester (Z-Pro-Ser-OMe, 171):

Z-Pro-OH + H-Ser-OMe.HCl $\xrightarrow{\text{Method VI}}$ Z-Pro-Ser-OMe (171)

Z-Pro-OH: (84%)

mp. : 79-80°C (lit.¹⁶⁸ mp. 78-80°C)

Z-Pro-Ser-OMe (171): (91%)

mp. : 104-105°C

ir : ν_{\max} (KBr) cm^{-1} : 3554 (NH), 3471 (NH), 3373 (NH), 3294 (NH), 1748 (ester), 1680 (carbamate), 1647 (amide I), 1558 (amide II), 1498.

nmr : δ ($CDCl_3$): 1.56-2.37 (4H, m, Pro $C^\beta H_2$ + Pro $C^\gamma H_2$), 3.53 (2H, t, Pro $C^\delta H_2$), 3.78 (5H, s+m, $COOCH_3$ + Ser $C^\beta H_2$), 4.22 (1H, m, Pro $C^\alpha H$), 4.53 (1H, m, Ser $C^\alpha H$), 5.12 (2H, s, Z CH_2), 7.34 (6H, m, Ser NH + aromatic protons).

ms : m/z: 351 (MH)⁺.

(11) Z-Pro- Δ Ala-OMe (172): (58%)

mp. : gummy

nmr : δ (60 MHz, $CDCl_3$): 1.66-2.40 (4H, m, Pro $C^\beta H_2$ + Pro $C^\gamma H_2$), 3.43 (2H, t, Pro $C^\delta H_2$), 3.76 (3H, s, $COOCH_3$), 4.33 (1H, m, Pro $C^\alpha H$), 5.10 (2H, s, Z CH_2), 5.70, 6.46 (1H, 1H, s, s, = CH_2), 7.20 (5H, s, aromatic protons), 8.73 (1H, brs, Δ Ala NH).

ms : m/z: 333 (MH)⁺.

(177) Reaction of Z-Met-Ser-OMe (25) with $(\text{COCl})_2$: Isolation of N-Benzyl-oxycarbonyl L-Methionyl- Δ Alanine Methyl Ester (Z-Met- Δ Ala-OMe, 173):

(i) Z-Met-Ser-OMe (25): experiment No. 13.

(ii) Z-Met- Δ Ala-OMe (173): (62%)

mp. : syrup

ir : ν_{max} (KBr) cm^{-1} : 3360 (NH), 1810, 1718 (br, ester), 1505 (amide II), 1435.

nmr : δ (60 MHz, CCl_4): 2.03 (3H, s, Met S- CH_3), 2.46 (4H, m, Met C^βH_2 + Met $\text{C}^\gamma\text{H}_2$), 3.80 (3H, s, COOCH_3), 4.50 (1H, m, Met C^αH), 5.06 (2H, s, Z CH_2), 5.30, 5.80 (1H, 1H, s, s, $=\text{CH}_2$), 6.56 (1H, brs, Met NH), 7.41 (5H, s, aromatic protons), 8.40 (1H, brs, Δ Ala NH).

ms : m/z: 367 (MH)⁺.

anal: Found: C, 55.44; H, 6.38; N, 7.29 %

Calc. for $\text{C}_{17}\text{H}_{22}\text{N}_2\text{O}_5\text{S}$: C, 55.74; H, 6.01; N, 7.65 %

(178) Reaction of Bz-Leu-Ser-Leu-OMe (105) with $(\text{COCl})_2$: Isolation of N-Benzoyl L-Leucyl- Δ Alanyl-L-Leucine Methyl Ester

(Bz-Leu- Δ Ala-Leu-OMe, 174):

(i) Bz-Leu-Ser-Leu-OMe (105): experiment No. 61.

(ii) Bz-Leu- Δ Ala-Leu-OMe (174): (30%)

mp. : syrup

ir : ν_{max} (KBr) cm^{-1} : 3320 (NH), 1773, 1741 (ester), 1632 (br, amide I), 1525 (br, amide II).

nmr : δ (CDCl_3): 0.87 (12H, br, Leu $\text{CH}_3 \times 4$), 1.65 (6H, m, Leu $\text{C}^\beta\text{H}_2 \times 2$ + Leu $\text{C}^\gamma\text{H}_2 \times 2$), 3.71 (3H, s, COOCH_3), 4.09-5.21 (4H, m, Leu $\text{C}^\alpha\text{H} \times 2$ + $=\text{CH}_2$), 7.00-8.06 (7H, m, Leu $\text{NH} \times 2$ + aromatic protons), 8.75 (1H, br, Δ Ala NH).

ms : m/z: 432 (MH)⁺.

anal: Found: C, 64.01; H, 7.34; N, 9.56 %

Calc. for $\text{C}_{23}\text{H}_{33}\text{N}_3\text{O}_5$: C, 64.04; H, 7.66; N, 9.74 %

(179) Reaction of Bz-Ala-Ser-Ala-OMe (107) with $(\text{COCl})_2$: Isolation of N-Benzoyl L-Alanyl- Δ Alanyl-L-Alanine Methyl Ester (Bz-Ala- Δ Ala-Ala-OMe, 175):

(i) Bz-Ala-Ser-Ala-OMe (107): experiment No. 62.

(ii) Bz-Ala- Δ Ala-Ala-OMe (175): (25%)

mp. : syrup

ir : ν_{max} (KBr) cm^{-1} : 3345 (NH), 3290 (NH), 1740 (ester), 1690, 1623 (br, amide I), 1525 (amide II).

nmr : δ (CDCl_3): 1.53 (6H, m, Ala $\text{CH}_3 \times 2$), 3.75 (3H, s, COOCH_3), 4.71 (2H, m, Ala $\text{C}^\alpha\text{H} \times 2$), 5.40, 6.46 (1H, 1H, s, s, $=\text{CH}_2$), 6.84 (1H, m, Ala NH), 7.03-8.04 (6H, m, Ala NH + aromatic protons), 8.62 (1H, br, Δ Ala NH).

anal: Found: C, 59.11; H, 6.28; N, 12.11 %

Calc. for $\text{C}_{17}\text{H}_{21}\text{N}_3\text{O}_5$: C, 58.79; H, 6.05; N, 12.10 %

(180) Reaction of Z-Ser-Leu-Ser-OMe (71) with $(\text{COCl})_2$: Isolation of N-Benzylloxycarbonyl L-Seryl-L-Leucyl- Δ Alanine Methyl Ester (Z-Ser-Leu- Δ Ala-OMe, 176):

(i) Z-Ser-Leu-Ser-OMe (71): experiment No. 40.

(ii) Z-Ser-Leu- Δ Ala-OMe (176): (30%)

mp. : syrup

ir : ν_{max} (KBr) cm^{-1} : 3382 (NH), 3322 (NH), 1784, 1719 (ester), 1648 (amide I), 1524 (amide II).

nmr : δ (CDCl_3): 0.88 (6H, brs, Leu $\text{CH}_3 \times 2$), 1.71 (3H, m, Leu C^βH_2 + Leu C^γH), 3.75 (5H, s+m, COOCH_3 + Ser C^βH_2), 4.53 (2H, m, Ser C^αH + Leu C^αH), 5.06 (2H, s, Z CH_2), 5.84, 6.50 (1H, 1H, s, s, $=\text{CH}_2$), 7.28 (7H, s+m, Leu NH + Ser NH + aromatic protons), 8.37 (1H, brs, exchangeable with D_2O , Δ Ala NH).

anal: Found: C, 58.28; H, 6.93; N, 9.33 %

Calc. for $\text{C}_{21}\text{H}_{29}\text{N}_3\text{O}_7$: C, 57.93; H, 6.67; N, 9.66 %

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